AD717409

PROJECT SANGUINE

BIOLOGICAL EFFECTS TEST PROGRAM
PILOT STUDIES

FINAL REPORT

November 1970

prepared for

DEPARTMENT OF THE NAVY
NAVAL ELECTRONIC SYSTEMS COMMAND HEADQUARTERS
Washington, D. C. 20360

Contract No. NOOO -39-69-C-1572

138

PROJECT SANGUINE

BIOLOGICAL EFFECTS TEST PROGRAM
PILOT STUDIES

FINAL REPORT

William B. Coate, et al.

November 1970

prepared for

DEPARTMENT OF THE NAVY
NAVAL ELECTRONIC SYSTEMS COMMAND HEADQUARTERS
Washington, D. C. 20360

Contract No. N000-39-69-C-1572

HAZLETON LABORATORIES, INCORPORATED
A Subsidiary of TRW Inc.
P. O. Box 30
Falls Church, Virginia 22046

FOREWORD

The work described in this report was performed under Contract No. NOOO-39-69-C-1572 authorized under Project SANGUINE by the U. S. Navy, Naval Electronic Systems Command Headquarters. It presents the details of the design, construction, and operation of electric and magnetic field simulators used to provide extremely low frequency (ELF) electromagnetic environments for various biota. Also it presents the details of a series of pilot laboratory studies (and one field study) on these biota. The studies were designed to determine whether the electromagnetic fields from an operational SANGUINE system could be expected to effect biota in its vicinity. The field strengths studied in the laboratory were well in excess of those expected from an operational SANGUINE antenna. It was anticipated that if effects were obtained at these field strengths, further research would be required to define their thresholds.

Project Office Insert

The primary purpose of these initial studies is to determine which, if any, potential problem areas warrant longer term, more intense study. If research efforts now underway reveal the need for subsequent studies, they will be pursued concurrently with other Project SANGUINE research and development efforts now scheduled through 1974.

In reviewing these reports, it should be noted that the electric (E) and magnetic (B) field levels at which these studies were conducted are substantially higher than those which would be associated with the operation of a SANGUINE system. Conceptual SANGUINE Systems now under study will operate at or below the level of 0.069 volts per meter above the antennas as opposed to the experimental values of 10 and 20 volts per meter and 0.2 gauss as opposed to the experimental values of 1 and 2 gauss.

W. K. HARTELL
CDR CEC USN
SANGUINE Division Director

DIGEST

This report contains a detailed presentation of (a) the design, fabrication, and technical operation of electric and magnetic field generators designed to provide laboratory simulation of SANGUINE-type extremely low frequency (ELF) electric and magnetic fields; (b) the methodology and results of a series of pilot studies performed in these fields; and (c) the methodology and results of a pilot field study of soil organisms in the vicinity of the Wisconsin Test Facility. The laboratory studies were conducted separately with 45 Hz and 75 Hz fields. The field strengths used in these studies were electric fields of 10 and 20 volts per meter and magnetic fields of 1.0 and 2.0 gauss. In some studies the electric field was set up in a conductive medium (moist soil, water, or nutritive bacterial medium) and in others in air. In one study, ELF current (0.5 mA) was passed through the body of the animals while they were in a combined electromagnetic field.

The laboratory studies consisted of the following, with data suggesting effects as shown:

- Rat Fertility Studies - no effects
- Canine Physiology Study - possible but inconsistent blood pressure and rectal temperature elevations
- Insect Mutagenesis Study - possible increase in dominant lethal mutation rate
- Bacteria Mutagenesis Study - no effects
- Plant Cytogenetic Study - no effects
- Seed Germination and Early Growth Study - possible growth inhibition in one of three species
- Multiple-Species Electrical Field Perception and Preference Studies - no preference effects except in the few turtles which reacted to the onset of 20 v/m fields. Fish reacted to the onset of 10 and 20 v/m fields.
- Rat Avoidance Learning Study - no effects

The field study showed declines in the populations of four major classifications of soil arthropods at both the test and control. No conclusive conclusion could be drawn from the data.

TABLE OF CONTENTS

CHAPTER A

INTRODUCTION

1.0 GENERAL OBJECTIVE

The purpose of this test program was to obtain data with which to assess preliminarily whether effects on biota in the vicinity of an operational SANGUINE system could be expected as a consequence of their exposure to electric and magnetic fields and earth return currents. Since the SANGUINE system would operate at extremely low frequency (ELF) and on the magnetic loop antenna principle with deep earth return currents, electromagnetic fields would be present in, on, and above the surface of the earth and bodies of water in its vicinity. Because so little is known about the thresholds for biological effects of prolonged exposure to electric or magnetic fields, or imperceptible current, at ELF, even at power frequencies (50 and 60 Hz), it was deemed necessary to begin a research program to determine the electromagnetic limits within which a SANGUINE system should be operated to ensure its compatibility with its biological environment.

2.0 SPECIFIC OBJECTIVES

Hazleton Laboratories, Inc., undertook (a) to design and perform laboratory studies to determine whether specified EM field and current strengths at 45 and 75 Hz in conductive or nonconductive media would have selected biological effects and (b) to conduct a census study of selected soil organisms in the vicinity of a ground terminal of the test antenna at the Wisconsin Test Facility before and after power-on operations were begun.

3.0 ELECTROMAGNETIC FIELD AND BODY CURRENT LEVELS

3.1 - At the outset of this project, the following field strengths were selected for test purposes at both 45 and 75 Hz:

- Electric fields: 63 volts per meter
630 volts per meter
- Magnetic fields: 1.0 gauss
10 gauss

3.2 - Prior to the start of any of the biological studies, but after design and fabrication of laboratory electric and magnetic field

generating systems, the following parameters were designated for testing at 45 and 75 Hz:

- Electrical fields: 10 volts per meter
  20 volts per meter

- Magnetic fields: 1.0 gauss
  2.0 gauss

- Body current: 0.5 milliamperes

3.3 - The rationale for the final values selected was as follows:

- SANGUINE system design studies had demonstrated that system performance requirements could be met with antenna currents which would (a) hold electrical field strength above the antenna cable to less than 0.1 volt per meter and (b) hold magnetic field strength above the cable to 0.2 gauss.

- Voltage gradients in the earth at the antenna grounds could be reduced by distribution to any values required for biological safety.

- Organisms in electrical contact with a current carrying natural medium, i.e., moist earth or water, will be subjected to body currents as a function of the potential difference across their bodies and their contact and body resistances. Larger organisms are thus likely to experience higher currents in a conductive environment containing a voltage gradient.

- Body current of 0.5 mA at power frequencies (ELF) is near the human perception threshold for momentary contact. An animal would be exposed to this current level if subjected, for example, to a 1.0 volt drop across a 2000 ohm total resistance. If the animal did not perceive the current, it might remain in the area indefinitely. No data exist for prolonged exposure to ELF at this current level.

4.0 FORMAT OF TECHNICAL REPORTS

Because the various studies were performed independently at different, but sometimes overlapping, times during the course of the contract period, each study is reported as an entity in a separate chapter separately paginated. Description of the apparatus used to provide the electromagnetic environment(s) is presented first. Special apparatus is described in the methods section of each chapter as pertinent.

Following the first 10 chapters, in each of which there is a discussion section, there is a general conclusion in which problem areas are highlighted. Recommendations for future research are made in a final chapter. These recommendations are not meant to be exhaustive but rather indicate areas in need of additional investigations based on results obtained in the present project.

CHAPTER B
ELECTROMAGNETIC FIELD SIMULATORS

Robert E. Kimball

TABLE OF CONTENTS

1.0 INTRODUCTION

Chapter B contains a detailed presentation of the design, fabrication, and technical operation of the electromagnetic field simulators installed at Hazleton Laboratories in fulfilling the requirements of the Naval Electronics Systems Command, Contract No. N000-39-69-C-1572. The objective of this contract was to assess preliminarily the possibilities of biological/ecological impact from the continuous presence in a given locale of extremely low frequency (ELF) electric fields, magnetic fields, and earth return currents of specified magnitudes. To this end it was required that these electromagnetic phenomena be generated in the laboratory in a manner appropriate to simulate up to 10 times "worst case" conditions for the given locale.

2.0 SUMMARY

Electromagnetic field simulators were designed, fabricated, installed, and operated in the laboratory so as to permit controlled exposures of a variety of biota to ELF magnetic fields and/or to electric fields in or directly on conductive or non-conductive media. The list of biological materials intended to be so exposed included cows, bulls, dogs, rabbits, rats, ducklings, fish, turtles, fruit flies, seeds, seedlings, and bacteria. The larger animals were to be fewer in number, the range being from four cows or bulls at a time, through scores of rats, hundreds of seeds, to thousands of bacteria. Exposures were to range in continuous duration from 24 hours for bacteria to over 100 days for rats.

The following characteristics were specified initially:

a. The electric and magnetic field strengths shall be held to 63 ± 5% volts per meter and 1.0 ± 15% gauss, respectively, or to appropriate multiples per design.

b. Exposures shall be at one and 10 times the basic EM field strengths for smaller biota and at one and two times the basic electric field strength for cows and bulls.

c. The resistivity of the contents of terrestrial material in or on which biota are exposed shall be held between 2000 and 5000 meter-ohms.

d. The conductivity of the upper stratum of terrestrial material shall be greater than that of the lower at all times.

e. Both 45 Hz and 75 Hz shall be studied.

Subsequently, prior to biological testing, the EM field strengths to be studied were set at 10 and 20 ± 5% volts per meter and 1.0 with 2.0 ± 15% gauss, respectively. In addition, certain studies were to be conducted using controlled 0.5 mA body currents in addition to exposure to airborne electric fields.

The small-animal EM field simulators generally functioned per design. Mechanical failures in the rotary generators providing the 75 Hz fields were the main source of difficulty. The large-animal E-field simulator never performed according to design and was abandoned in favor of body-current to be provided by attached electrodes. This latter system functioned well but the studies were eliminated on other technical grounds.

3.0 TECHNICAL

3.1 Introduction

Initially, design criteria were provided by the Navy which specified that the maximum electrical gradient at the earth's surface could be as high as 63 volts per meter and the magnetic field as high as 1.0 gauss, and that tests were to be conducted at one and 10 times these values with smaller biota and at one and two times the electric field value with cows and bulls. It was further specified that tests on terrestrial animals were to be conducted in such a way that body currents resulting from step potentials be present. In order to meet this requirement, the electric field had to be horizontally oriented in a conductive medium for certain tests. Later, the electric field strength criterion was reduced to 10 volts per meter and the multiple for smaller biota reduced from 10 to two times the 10 volts per meter and 1.0 gauss values. Further, it was finally decided to pass controlled body currents through certain animals rather than rely on contact with a conductive substrate to create step potentials of fixed values.

The above requirements comprised the technical constraints on simulator designs. Economic constraints were also present. Thus, for example, electronic generation of the fields, while technically to be preferred was not economically feasible because of the very high power requirements inherent in the original design criteria.

In the subsequent subsections of this section, distinction will be made between the simulation facility developed for EM field exposures of the smaller biota - henceforth called the small-animal facility - and the facility developed for electric field and body current exposure of cows and bulls - henceforth called the large-animal facility.

3.2 Small-Animal Facility

3.2.1 Physical Layout

The small-animal facility was located on the ground floor of Building 19 of the Hazleton Laboratories at 9200 Leesburg Pike, State Route 7, west of Falls Church, Virginia. The room layout is shown in Figure B1. Three rooms were devoted to installation of the test fixtures. The power generators were located together in a separate room.

3.2.2 Test Fixtures

The test fixtures each consisted of a table, 2' - 6 1/4" high, 15' - 0" long, and 4' - 6" wide, upon which rested seven wooden boxes (Figure B2). The inside dimensions of each box were 120 cm long, 50 cm wide, and 30 cm deep. Each box was divided into six cells by planar electrodes, spaced 20 cm apart. The electrodes are described below in Section 3.2.3. Plexiglas® partitions parallel to the box sides were located 10 cm from each long side. These partitions were designed primarily to establish electrode spacing and to restrict the surface area under terrestrial animals to 30 x 20 cm, thereby avoiding anticipated electrical edge effects. It turned out that edge effects (due to the presence of the wood electrically in parallel with a low conductivity substrate soil) could best be avoided by filling only the 30 x 20 cm cell. Wooden spacers were then placed between the Plexiglas® partitions and wooden box sides to prevent bulging of the plastic. No ferrous metal was used in construction of the test fixtures.

Cages (30 x 40 x 20 cm) for the small terrestrial animals were designed to fit directly over the 30 x 20 cm cells. Each cage spanned two cells and had a removable center partition, thus allowing exposure areas of 30 x either 20 or 40 cm. Each box accommodated three cages. The cages were made of Plexiglas®. The original plastic tops were replaced with wood, since the plastic tops tended to warp and would have permitted animals to escape. Figure B3 shows the cages with test animal .

Initially, transparent cages were used. Later, cage sides and partitions were made opaque to minimize any unnatural visual "crowding" effect on contained rats.

3.2.3 Electric Field Design

The electric field was established between each pair of electrodes which divided the boxes into individual exposure cells. The electrodes, below the surface of the medium, were oriented to produce horizontally aligned fields. The electric fields were transverse to the long dimension of the table in order to be at right angles to the magnetic field (as will be described in Section 3.2.4). Originally, the electrodes were made up of horizontal rods of 1/8 inch stainless steel spaced 1.5 cm apart and welded at one end to a vertical rod of the same stock. This resulted in a structure resembling a comb with long tines. Stainless steel was selected to meet the following requirements:

- Poor magnetic material
- Electrical conductor
- Noncorrosive and nontoxic
- Retain shape
- Hard enough to resist chewing
- Ready availability

Spacing of the electrodes was maintained by the Plexiglas® partitions, which were drilled to accept the horizontal rods.

Later, after difficulties had been experienced with neonatal rats crawling through the electrode interstices into adjacent cells, galvanized hardware cloth with 3/8 inch mesh was fastened to each electrode for the remaining rat breeding studies.

Smaller versions of this design were employed in one study: onion bulbs were rooted in small glass boxes containing one pair of scaled down electrodes submerged in water.

Fruit flies were exposed to airborne electric fields established between pairs of electrodes in empty cells. Dogs similarly were exposed to E-fields established between pairs of electrodes approximately two feet above table top height.

3.2.4 Magnetic Field Design

The magnetic coil system was designed specifically for 45 and 75 Hz. It was designed to produce a field of up to 10 gauss (rms) with an uniformity of ±15% over a region 3.5 meters long by 0.5 meters high and 1.2 meters deep. The total height (and width) of the system was 6 feet 5 inches. The input current could not exceed 12 amperes. The power supply voltage could not exceed 115 volts.

A scale model of the system was set up in the laboratory and the design, as it progressed, was checked out empirically. The concept is shown in Figure B4.

The realized four-coil system yielded a region of approximately 3.5 m x 1.2 m x 1.2 m with a uniformity of ±10%, somewhat better than that specified. The spacing between the coils was 1.0 m between the two center coils and 1.2 m between each end coil and its neighboring center coil.

In order to keep the input current equal to or below 12 amperes, it was necessary to add capacitance to the circuits. The capacitance could have been added either in series or in parallel with the coil system.

The series configuration had the following parameters:

Frequency Hz	I Amperes	R Ohms	Turns No.	ωL Ohms	$\frac{\omega L}{R}$	C µf	Vc Volts
75	10	12	90	212	17.7	10	2120
45	10	12	90	127	10.6	28	1270

The parallel configuration had the following parameters:

Frequency Hz	Volts	I Amps	Turns No.	ωL Ohms	R Ohm	i Amps	C µf	Bus Size Sq. Inch
75	97	10	4	0.43	0.019	225	4900	0.35
45	65	10	6	0.58	0.052	113	6100	0.20

The capacitance for the parallel case needed to withstand only the generator voltage. Thus, 236-volt, 50 µf commercial motor-starting capacitors could be used.

The bus size was calculated for hard-drawn aluminum. In the final design, 2 x 1/4" aluminum bar stock, having a cross-section of 0.5 square inch, was used for both frequencies.

The parallel connection was chosen because large voltages are not generated. This choice had the disadvantages of making the system sensitive to harmonic content of the power supply and of requiring careful fabrication practices to keep the total coil resistance down. The coil cross-section was made square, 77 inches on the size, to make the fabrication easier and more economical. Lap joints were used at the corners which, with 1/4 inch stock, gave a coil length of about 1 inch per turn. The aluminum bars were cut to length, the overlaps polished, coated with commercial aluminum joint compound, and bolted together. Welded joints were considered but not used since the bolted method gave a workable system, although the resistance was slightly greater (and hence coil Q lower) than design.

The tuning capacitors were wired to the system with 3-0 copper welding cable. Aluminum bus was considered, but the cable was preferred, due to its flexibility. The capacitors actually used were banks of commercial transmission line power factor correction capacitors. These capacitors are in 1237 μf units. Four units made up the 4900 μf for the 75 Hz system, five the 6100 μf for 45 Hz. Final tuning was done with commercial a-c motor-starter capacitors wired in parallel.

The systems were tuned by measuring the field strengths and searching for a maximum as a function of capacitance. This method was felt preferable to measuring the inductance of the system as built, then adding the capacitance and making a final adjustment by minimizing the generator current.

The fabricated systems had a poorer Q than design, but no attempt was made to upgrade the Q since the experimental requirements were subsequently changed, for other reasons, so as not to require a 10 gauss field. The 2.0 gauss maximum finally specified was obtained readily with the poorer Q and with the system not tuned precisely.

The design values of 4900 and 6100 µf proved significantly low; the capacitances actually used were as follows:

Gauss No.	Frequency (Hz)	Capacitance (µf)
1.0	45	6948
2.0	45	7238
1.0	75	7785
2.0	75	7785

The fields were mapped using a probe coil built and calibrated in the laboratory. The probe coil constant was 8.6 µv per gauss Hz, giving 0.39 mv for 1 gauss at 45 Hz and 0.645 mv at 75 Hz. The probe coil had a measured inductance of 2.6 mh and resistance of 3.0 ohms. The voltage values were measured across a 100K resistance.

The fields were remarkably uniform within the volume used for exposure of biota, varying less than ±10% from nominal value.

The magnetic systems were supplied power through variable autotransformers, protected by fuses. Once calibrated, the field values could be set by measuring the coil current with an Amprobe snap-around ac voltage/current tester or by measuring the input current from the power supply. The former was our practice after calibration on the latter.

The coils were hung from the room ceilings by means of a wooden frame which in turn was suspended below an overhead track by aluminum and nylon traveler wheels. This arrangement permitted the coils to be moved along the tables sufficiently to allow ready access to all boxes for cleaning, animal feeding, etc. (Figures B2 and B3).

The magnetic field systems were remarkably trouble-free and reliable in operation.

3.2.5 Power Supplies

The requirement to test at both 45 and 75 Hz necessitated procurement of special power supplies. To produce fields of 630 v/m and 10 gauss in soil or water of less than 5000 meter-ohms meant that these power supplies had to be capable of supplying up to about 6 kw per frequency. This value includes

2 kw for the electric fields and 4 kw for the magnetic fields. Power losses in the ground due to the electric field were calculated as follows:

$$P = E^2 \sigma$$

$$= (63\tfrac{v}{m})^2 \times 5 \times 10^{-4} \tfrac{mho}{m}$$

$$= 1.98 \text{ watts}/m^3$$

Volume of a small-animal soil box:

$$0.3 \times 0.5 \times 1.2 = 0.18 \text{ m}^3$$

Total volume for each frequency:

$$2 \times 7 \times 0.18 = 2.52 \text{ m}^3$$

Total power for each frequency:

$$2.52 \times 1.98 = 4.99 \text{ watts @ } 63 \text{ v/m}$$

At 10X, or 630 v/m, the total power would be:

$$100 \times 4.99 = 499 \text{ watts}$$

Because of uncertainties in the ground resistivity, a safety factor of about 4 was used. Consequently, 2 kw was specified for each frequency.

Specific values of the power supply ratings for the magnetic field simulators were determined more or less arbitrarily to get a combination of voltages and currents which would give a reasonable coil assembly design and still be obtainable from generally available components. The specific values selected were 115 v, 12 amps per coil system.

A fundamental decision was the type of power supply to use: electronic or rotary. The factors considered were as follows:

a. Technical capability

- Voltage
- Current
- Frequency
- Waveform

b. Nontechnical factors

- Availability of components
- Convenience in using
- Cost

Electronic generators, i.e., oscillators with amplifiers, were considered. While they have advantages such as operational convenience, quiet operation, and the capability of being modulated (not a requirement), their high cost was the controlling factor against using them. (After the requirement for the maximum E-field was changed from 630 v/m to 20 v/m, and the maximum H-field from 10 to 2.0 gauss, the electronic generators might have been competitive.) As will be seen later, electronic power supplies were used for body-current experiments.

Rotary machines, i.e., dynamo-type generators, specifically designed and built for 45 and 75 Hz would have caused prohibitive expense and delay. However, commercially available oversped or undersped 60 Hz machines were not so costly. This use of 60 Hz rotary machines was discussed with generator manufacturers. The consensus was that underspeeding should be no problem, assuming that the generator was separately excited to get the proper voltage. One manufacturer did say that if a generator were run too slowly, one might have a problem with harmonics. On the other hand, overspeeding the generator was not recommended as a standard practice but was reasonable as a laboratory procedure. Again, the field currents would have to be adjusted to get the proper output voltage. The speed should not exceed 125% rated speed for reasons of mechanical safety. Even at 125% speed (corresponding to 75 Hz for a 60 Hz generator), brush, commutator, and bearing wear would be accelerated. The manufacturers pointed out that the AC generators of this size are subject to about 10% harmonic distortion. The manufacturers did not know the frequency distribution of the distortion.

It was specified* that 10% harmonic distortion was unacceptable and that filters would have to be used. Since filters at these powers and frequencies

---

* Verbal instruction from H. N. Cantor, IIT Research Institute

were not available "off-the-shelf," low-pass filters were designed with a cut-off between the fundamental and the second harmonic. The parameters selected are given in Figure B5. It turned out that the predominant harmonic was the 24th,* so that the much simpler filter shown in Figure B6 could be used. (The capacitors thus rendered surplus were used as part of the tuning capacitance needed for the magnetic field coil systems.)

Customary practice with small generators is to use prime movers rated at about 2.5 hp per kw of generator output. The machines selected for each frequency were as follows:

Generator for E-field:

Dayton Model 1W546, rated 2 kw, 115 v, 3600 rpm

Generator for H-fields:

Dayton Model 1W628, rated 3.6 kw, 115/230 v, 3600 rpm

Prime-Mover

Dayton 15 hp Motor, 3-phase, 208 v, 1800 rpm

The two generators were connected by a flexible coupling, and the pair belt-driven by a variable-speed drive consisting of a T.B. Wood's Son Model MS127 variable pulley on the motor and a companion flat pulley on the larger generator.

The variable pulleys were adjusted to drive the 45 Hz generators at 2700 rpm and the 75 Hz generators at 4500 rpm. The speeds were set roughly using a Zero-Max portable tachometer; fine adjustments were made as needed using special Frahm frequency meters installed in the generator output circuits.

The generator sets were located in a building equipment room, separate from the animal exposure rooms to minimize noise interference and facilitate maintenance. The motors were wired to building circuits which were switched to the stand-by emergency power plant through automatic switching when the commercial power is interrupted. Standard wiring practices, according to national and local code, were used.

---

* The harmonic is related directly to the number of slots in the armature.

The generator outputs had fused disconnects. The filters were located at the generators on the load size of the disconnects. The feeder circuits to the animal rooms had standard toggle switches just inside the entrances to the exposure rooms so that technicians could shut off the power from the rooms as necessary.

3.2.6 Controls

Each exposure room was equipped with a panel on which were mounted the switches, variable transformers, fuses, and meters used to control the electric and magnetic fields. The electric field of each soil box consisting of six exposure cells could be adjusted independently to the desired level. Within each box, each cell could be switched on or off. However, the 42 cells (seven boxes of six cells) within each coil system were necessarily exposed to the same magnetic field. Figure B7 is a simplified wiring diagram of the panel and Figure B8 a general view.

3.2.7 Problem Areas: Potential and/or Actual

3.2.7.1 Overspeeding

The primary problem was in the mechanical insults to the generators running at overspeed. These were manifested by extreme sensitivity to shaft alignment, excessive brush wear, and susceptibility of bearings to damage. This problem was minimized by careful alignment of the equipment and frequent inspection of high-wear parts. The use of harder brushes was considered, but rejected so as not to aggravate commutator and slip ring wear.

A second problem associated with off-speed operation was in adjustment of the field currents of generators. The generators used were self-excited machines designed for 3600 rpm operation. The 45 Hz generators, operating at 2700 rpm, required separate excitation which was supplied by a solid-state bridge rectifier with a simple R-C filter. Our apprehensions that the fields might overheat, since a higher-than-normal field current would be needed to obtain rated voltage at subrated speed, proved unfounded. However, the machines were operated at somewhat less than the rated voltage (115 v) to be sure the field windings were not subjected to significant overcurrent. They were very stable.

The 75 Hz generators, operating at 4500 rpm, originally were self-excited with external resistance added to reduce the field currents. The added external resistance introduced two problems:

(1) Difficulty in building up voltage upon starting
(2) Voltage instabilities

The first problem was circumvented by installing a momentary shorting switch across approximately half of the external resistance. The second condition was tolerated for a short while, but we then installed a separate exciter substantially the same as that used for the 45 Hz generators and abandoned the external resistances. This solved both problems.

3.2.7.2 Harmonics

Harmonics were present in the electric field systems only, since the tuned coil systems responded to the fundamental and not to any harmonics in the supply. The magnetic fields were thus essentially purely sinusoidal. In addition to the 24th harmonic introduced by the generator armature construction, the variable transformers in the system tended to saturate. The filters took care of the 24th harmonic, but careful operating procedures were required on the 45 Hz electric field system to be sure distortion was not introduced by the transformers. The resulting electric fields were nearly purely sinusoidal.

3.2.7.3 Soil Resistance

Initially we anticipated we would have trouble holding the soil resistivity within specifications, since the animals would urinate on and otherwise disturb the soil. Conductivity measurements made on various local soils gave wide ranges of values. A prototype cell was set up with rats and rabbits in the cages. It was extremely difficult to control resistivity using natural soils. Additionally, the soils were difficult to handle and very dirty. A series of experiments was made using granulated dried clay used to sop up oils from garage floors. We found that by using a

dry layer about 6 inches deep in the bottom of the box overlaid with about 6 inches of the material moistened to obtain the desired conductivity not only gave us the desired total resistivity but was remarkably constant over relatively long periods with the cages occupied. Furthermore, the material was relatively clean and easy to handle, and additional batches could be prepared with predictable resistivity. Resistivity in each cell of each box, including control boxes, was measured daily using a multimeter (VOM) to measure resistance which was compared with a range of permissible values calculated from the formula:

$$R = \rho \frac{l}{A}$$

where R is the resistance, l is the length of the cell, A is its cross sectional area, and ρ is the desired resistivity.

3.2.7.4 Field Uniformity

14

Field uniformity did not turn out to be a problem in the small-animal facility. Both the electric and magnetic fields were remarkably uniform throughout the exposure volumes. Close to the electrodes (i.e., within a centimeter or so), the electric gradients were higher by about 50% than throughout the rest of the volume when the crushed clay was in place. Once away from the grids, the electric fields were uniform. The E-field was uniform in water. The magnetic fields were essentially uniform throughout the exposure volume, divergences occurring only near the conductors and beyond the ends of the system.

The electric field was measured by inserting probes into the soil or water and measuring the voltage difference with a high impedence voltmeter (actually a calibrated oscilloscope). At these low frequencies, the tangential component of the electric field is essentially conserved at the surface, so the field in air very close to the soil interface would be practically the same as in the ground. The exposure volume of each cell was so very small compared to the wavelength of the electric field that the condition could be considered quasi-static.

The magnetic field was measured by a probe coil especially constructed and calibrated. The magnetic exposure volume was very large compared to the size of the sensing coil so that the act of measurement did not appreciably distort the field.

3.2.7.5 Interference

Due to space limitations, the coil systems had to be installed closer together than we would have preferred. However, there was no detectable mutual interference. Furthermore, no interference problems arose among the 45 Hz, 75 Hz, and 60 Hz house frequencies.

3.2.7.6 Safety

Since much of the simulators was necessarily exposed, electrical shock hazards could exist, at high voltages. The systems were designed, installed, fused, and grounded to minimize the probability of contact with high voltage. The exposure rooms were off-limits to other than project personnel and were kept locked when unattended. The generator area was fenced off from the rest of the equipment room and warning signs posted. Project personnel were briefed on the possible hazards involved and instructed to turn off power before touching the simulators.

3.2.8 Special Simulator Devices

3.2.8.1 Background

During the year following initiation of this contract, a number of developments led to the concept that in order to achieve "worst case" electric field impact on medium to large terrestrial animals, it would be desirable to pass known amounts of current through their bodies on a path similar to that involved during normal contact with the largest achievable "step potential." To accomplish this it was deemed necessary to attach electrodes to diagonally opposite front and rear legs and apply voltage from a constant current generator. The current magnitude selected for study was 0.5 mA. Exposures of E. coli bacteria to electrical current were made in special chambers as described in 3.2.8.4.

3.2.8.2 Constant Current Generator

In order to stabilize body current at 0.5 mA despite progressive but uncontrollable changes in contact and/or skin resistance, a device employing an LM201 operational amplifier (Figure B9) was interposed between the 45 or 75 Hz electric field source and the pair of electrodes to be attached to each animal. This device maintained constant current through the animal with total resistance between 0-10,000 ohms.

3.2.8.3 Electrodes

Electrodes of appropriate size for placement on dog's "ankles" were constructed of fine stainless steel. In use, the electrodes were placed on shaved areas which were covered with Burdick electrode paste.

Limp prod-wires were led from an overhead point to the electrodes. Adhesive tape was used to secure the wires to the dog during daily exposures to the body current.

3.2.8.4 Bacterial Culture Exposures

In order to expose E. coli bacteria to "worst case" electrical conditions, small Plexiglas® culture chambers were constructed. Each chamber was provided with low resistance (<400 ohms) electrodes prepared by mixing graphite in a fast-drying vehicle consisting of ethylene dichloride dissolved styrofoam which was painted on the interior ends of the 14.5 cm long chamber. A Nu-way® stud was previously threaded through each end and filed flush with the interior surface. Leads to the electrodes were brought from appropriate 45 or 75 Hz sources. Application of a 20 volt per meter gradient (i.e., 2.9 volts on the electrodes) resulted in between 4.4 and 5.0 mA current flow through the liquid medium containing the bacteria.

3.3 Large-Animal Facility

3.3.1 Earthborne E-Fields

Initially, it was planned to conduct preliminary studies on reproductive physiology in cows and bulls

exposed to earthborne electric fields. A facility was developed in which four ruminants were individually stanchioned while standing on a conductive medium (earth) in which electrodes were buried. Technical difficulties in achieving control of the resistivity of the substrate led to a design revision. The buried electrodes were disconnected from the generators.

3.3.2 Generators

Rotary 2 kw generators driven by 5 hp electric motors similarly to those used for the small-animal facility were used to supply the necessary power. Step-up transformers were used to obtain potential differences up to 1850 volts across air-spaced electrodes.

3.3.3 Airborne E-Fields

One-inch mesh hardware cloth electrodes, 4' x 6', were suspended at opposite ends of the animals' stalls isolated from ground. Electric field measurements without the animals showed a very steep gradient near the electrodes and essentially a zero voltage in the region where the animals would stand. It was deemed impractical to attempt to reach a 20-volt per meter voltage gradient across a cow with the generating equipment at hand.

3.3.4 Body-Current System

During the early course of this project, there had developed a growing concern over possible step potential hazards to large terrestrial animals (as distinguished from exposure to airborne electrical fields). Circuitry and electrode systems were developed to permit passage of up to 0.5 mA current via diagonally opposite fore- and hindlimbs. The constant-current device to regulate the current is shown in Figure B7.

3.3.5 Cancellation of Tests

The bovine studies were eliminated from the work scope, prior to initiation, on the basis of sample size and cost effectiveness. It was concluded that regardless of the results of the studies, the data could not be interpreted validly.

4.0 APPENDICES

4.1 Figures

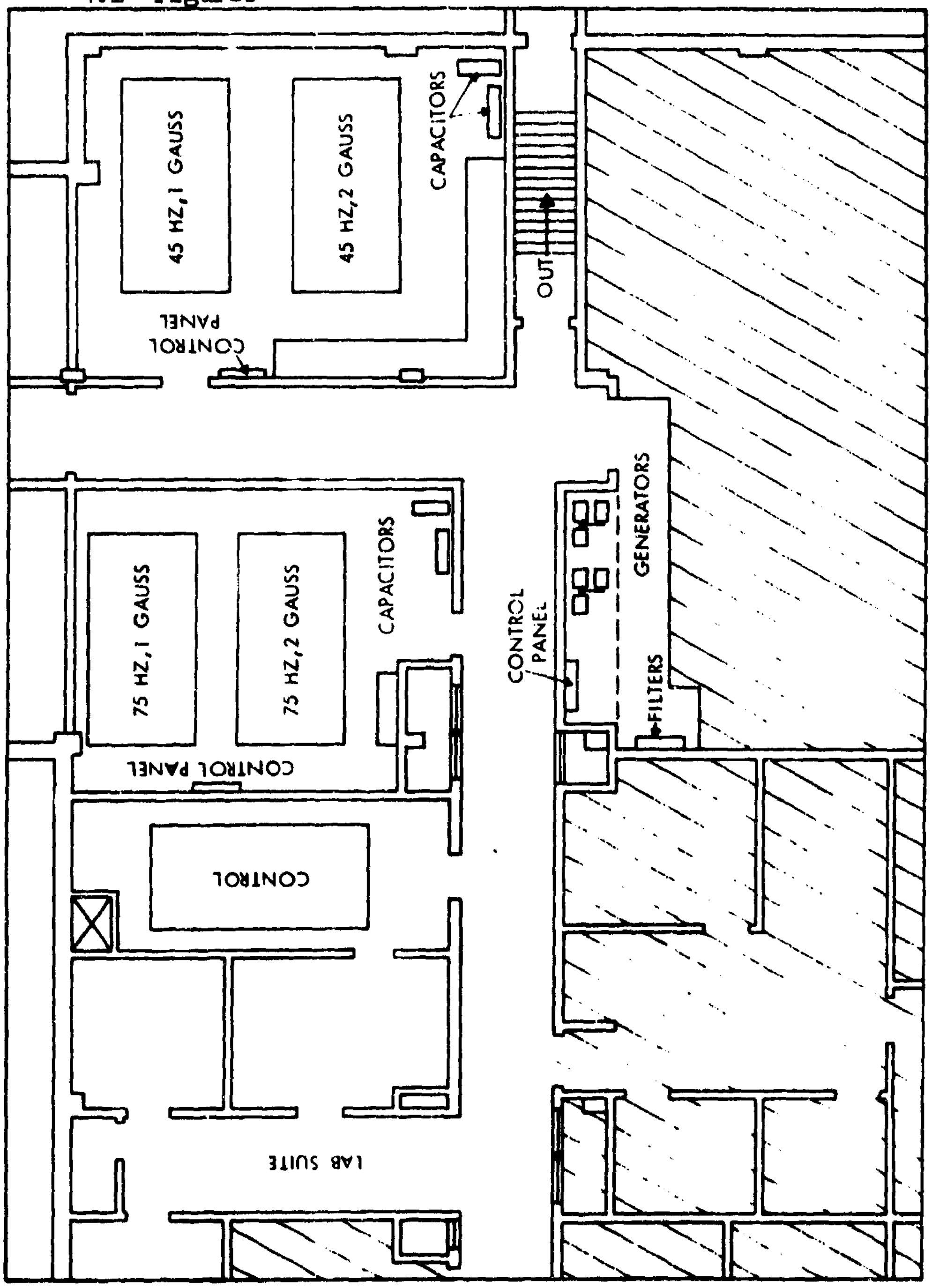


Figure B1 - SMALL-ANIMAL FACILITY

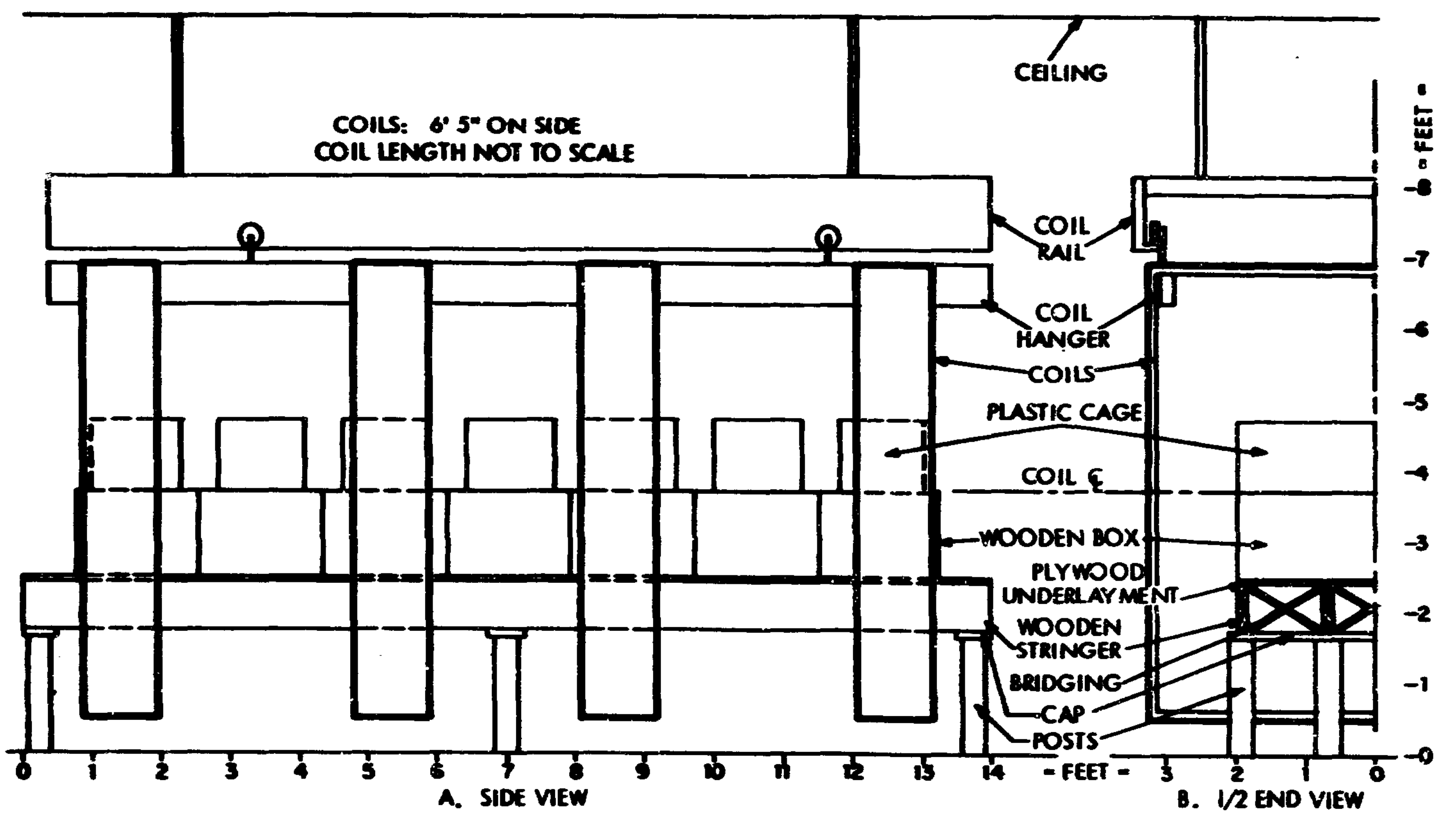


Figure B2 - SMALL-ANIMAL FACILITY
RELATIVE POSITION OF COILS TO EXPOSURE VOLUME

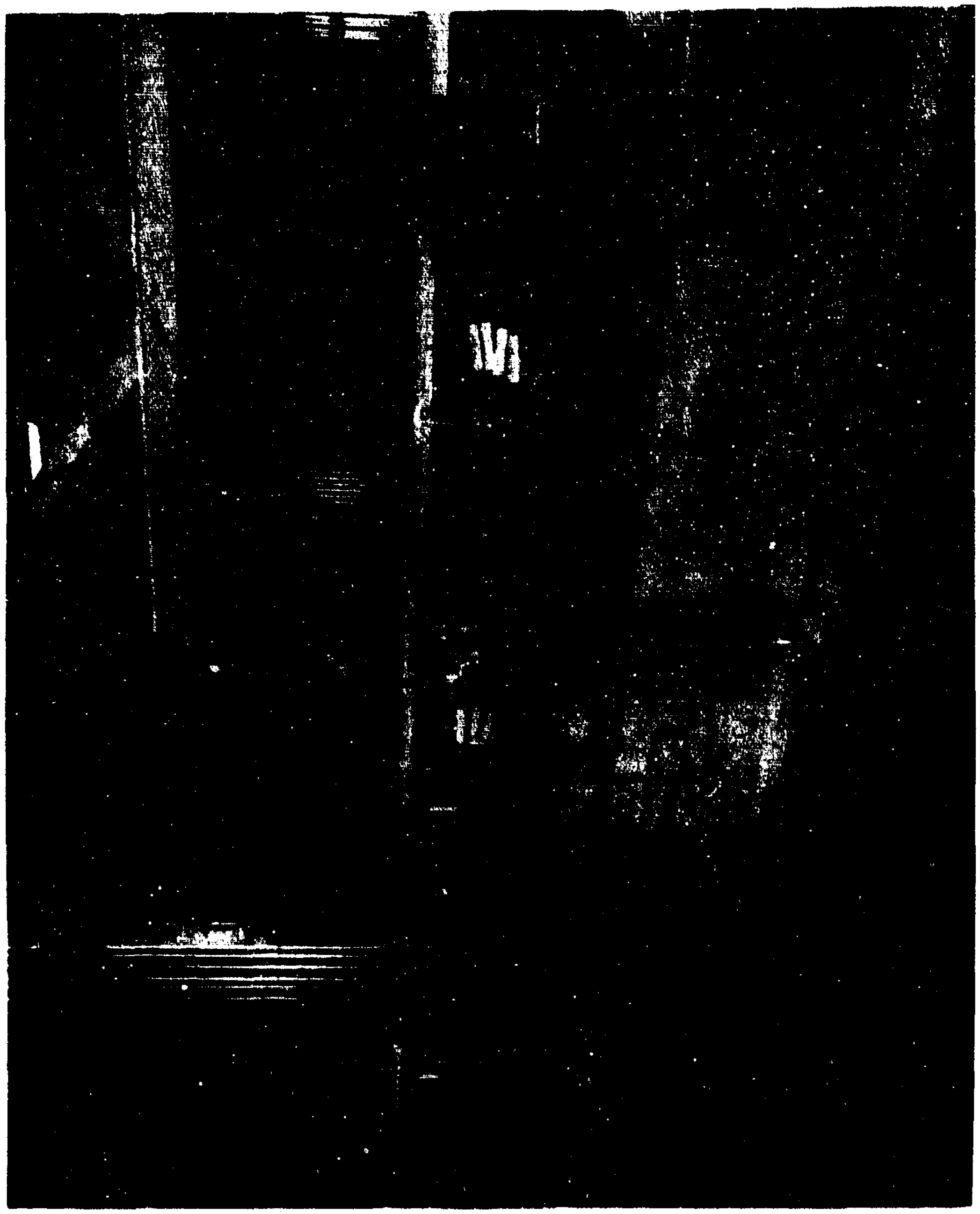


Figure B3 - SMALL-ANIMAL CAGES ON TEST STAND

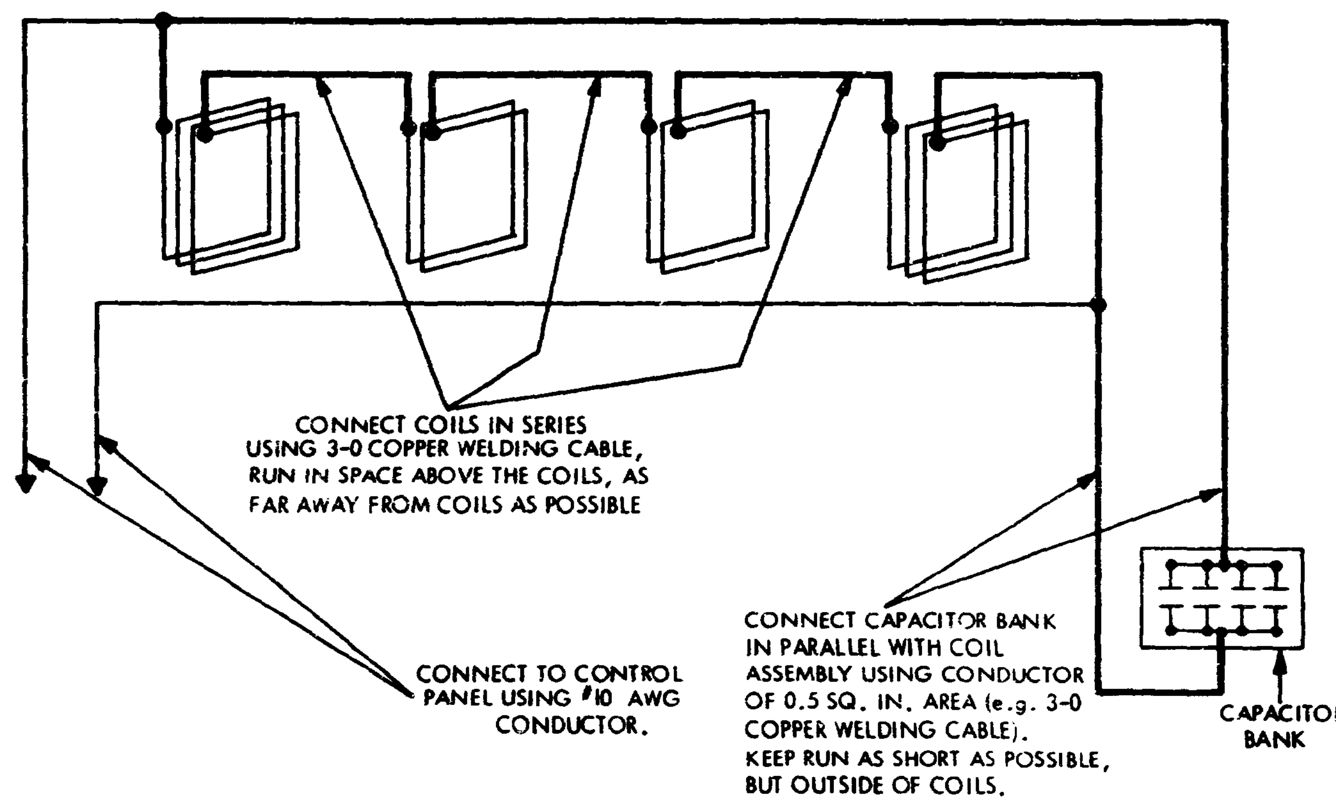
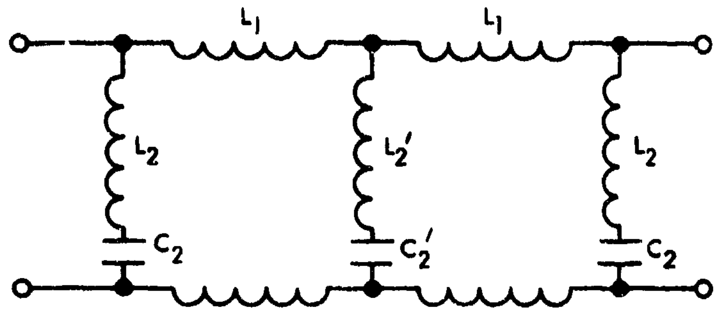


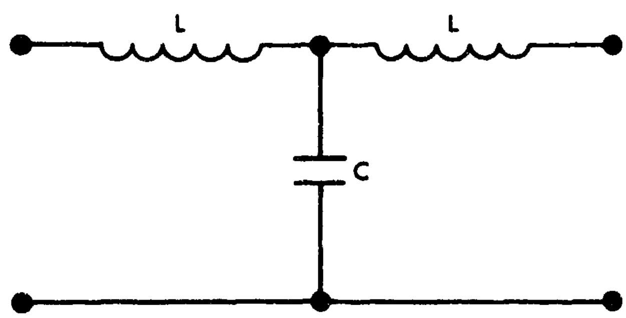
Figure B4 - CONCEPT FOR COIL ASSEMBLY



TYPE: LOW-PASS M-DERIVED

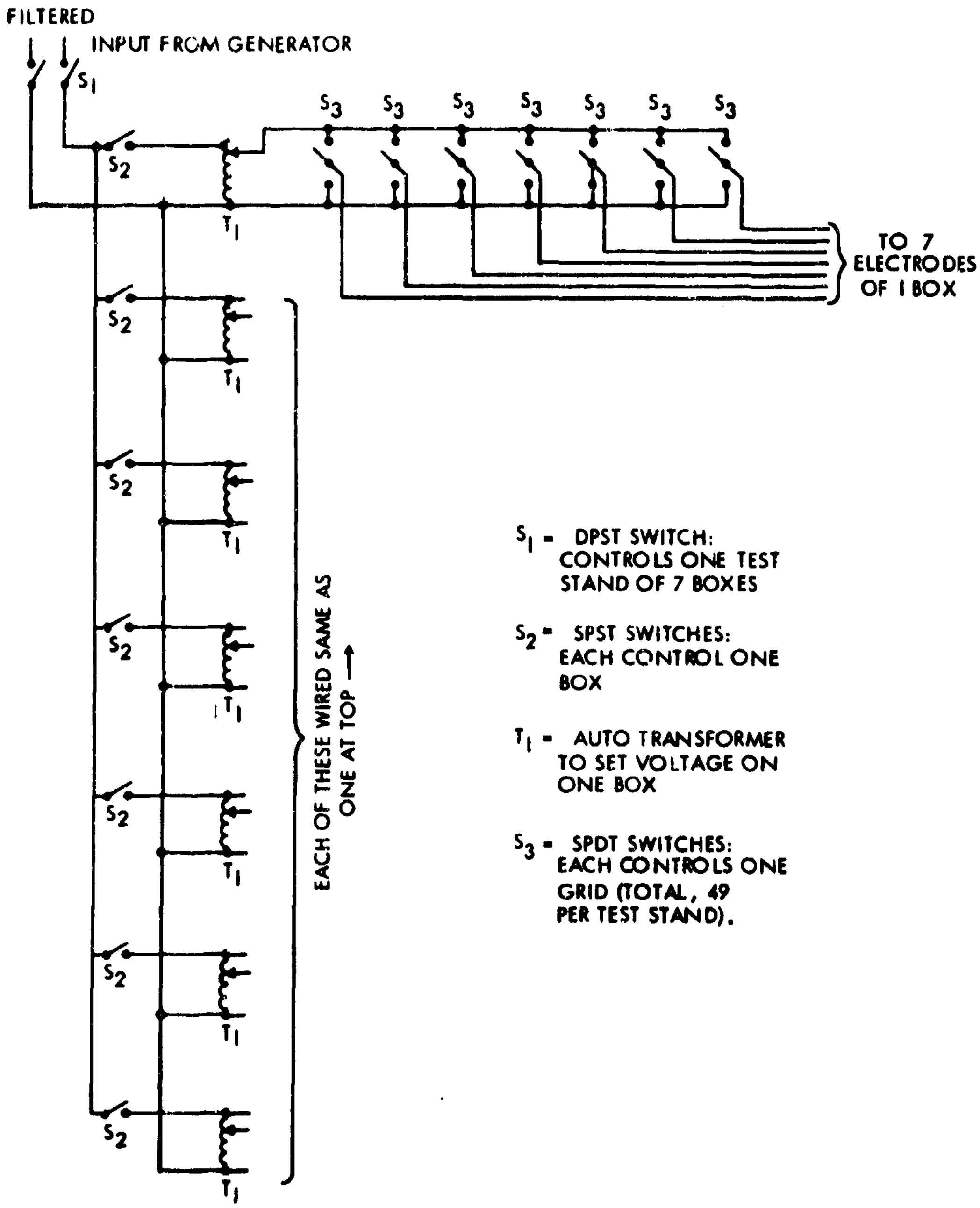
ELEMENT	UNIT	LINE FREQUENCY	
		45 HZ	75 HZ
L_1	mh	35	17.2
L_2	mh	24.5	12.3
L_2'	mh	1.67	1.3
C_2	µf	265	133
C_2'	µf	820	396
CUT-OFF	HZ	50	100
MAXIMUM ATTENUATION	HZ	135	225

Figure B5 - LINE FILTERS



FREQUENCY	L	C
45 HZ	35 mh	40μf
75 HZ	17 mh	25μf

Figure B6 - FINAL FILTER DESIGN



S_1 = DPST SWITCH: CONTROLS ONE TEST STAND OF 7 BOXES

S_2 = SPST SWITCHES: EACH CONTROL ONE BOX

T_1 = AUTO TRANSFORMER TO SET VOLTAGE ON ONE BOX

S_3 = SPDT SWITCHES: EACH CONTROLS ONE GRID (TOTAL, 49 PER TEST STAND).

Figure B7 - CONTROL PANEL SIMPLIFIED WIRING DIAGRAM

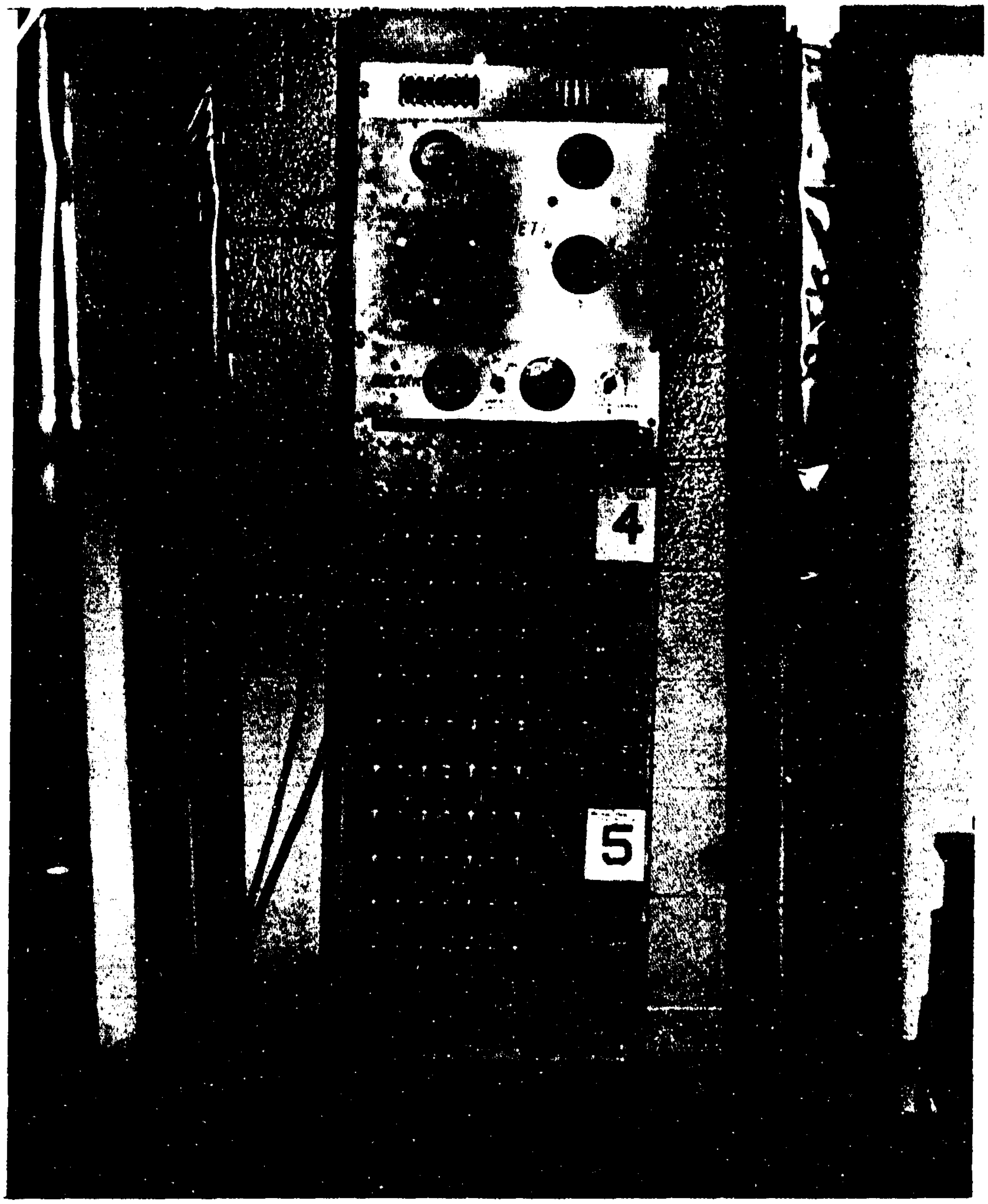
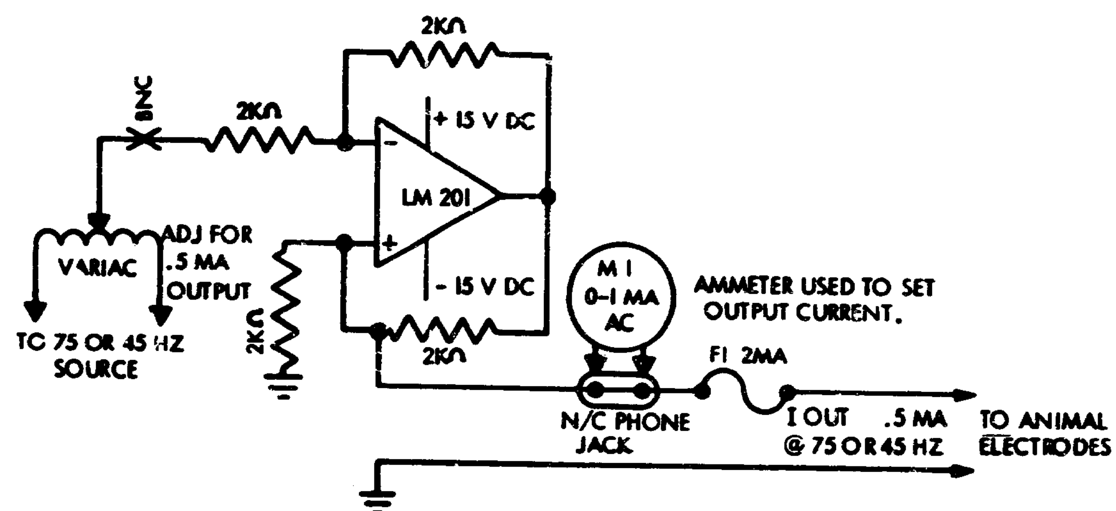


Figure B8 - GENERAL VIEW OF CONTROL PANEL - 75 HZ



NOTES: 1. ALL RESISTORS ± 1%
2. MAX LOAD RESISTANCE < 10 KΩ

Figure B9 - CONSTANT CURRENT GENERATOR

4.2 Glossary

Step potential - The electrical potential difference applied to an organism by virtue of its being in electrical contact at two discrete points on a voltage gradient (see Project SANGUINE Special Topic Memorandum No. 19, Step Gradient Theory and Bravo Test Facility, 17 April 1969, RCA Laboratories, Contract No. N000-39-68-C-1518).

CHAPTER C
RAT FERTILITY STUDIES

William B. Coate
Frederick E. Reno

TABLE OF CONTENTS

1.0 INTRODUCTION

Chapter C contains the methodology and results of three rat fertility studies performed in fulfilling the requirements of the Naval Electronic Systems Command, Contract No. N000-39-69-C-1572. The objective of this contract was to assess preliminarily the possibilities of biological/ecological impact from the continuous presence in a given locale of electric fields, magnetic fields, and earth return currents of specified magnitudes. The present studies were performed as pilot studies to determine whether gross effects on reproductive capability and/or early growth could be expected from these levels of electromagnetic (EM) fields and/or earth return currents at extremely low frequencies (ELF).

2.0 SUMMARY

Albino female rats were allowed to mate, gestate, deliver, and rear their offspring, on a dry-crushed clay substrate with 2000-5000 meter-ohms resistivity, while exposed to a 10-volt per meter or a 20-volt per meter electric field in the substrate and to either a 1.0 or 2.0 gauss magnetic field concurrently. Some offspring were reared to adulthood and mated to produce a second generation similarly exposed. Some females were sacrificed and Caesarean sections performed at 14 days of pregnancy and their uteri and fetuses examined. Histological examinations were made on adults exposed for over 100 days to the fields. When compared to controls similarly reared but without the ELF electromagnetic fields, no effects attributable to the EM fields were observed.

3.0 METHODS

3.1 Introduction

Rat reproduction studies have been widely used as a part of safety evaluations for drugs and food additives prior to human use. The present study, by encompassing the entire life cycle, was designed to permit the EM fields to impact reproductive potential at any point.

3.2 Materials

One hundred and sixty female and 80 male Sprague-Dawley rats obtained from Charles River Laboratories plus 100 females and 50 males derived from them were used in these studies.

3.3 Apparatus

The details of the simulation apparatus used to generate and deliver the electromagnetic fields are contained in Chapter B of this report. In the present study, rats were caged, bred, and reared in open-bottomed Plexiglas® cages, with plywood tops, set directly over 20 x 30 x 30 cm deep cells containing dry-crushed clay moistened to have a resistivity of 2000-5000 meter-ohms. The top half of the substrate had lower resistivity than the bottom half. The 20 cm sides of the cages set edgewise on Plexiglas® spacers which held stainless steel rod electrode arrays which formed the 30 cm ends of the cages beneath the substrate. Figure 1 shows a rat standing on the substrate in its 20 x 30 cm cell on the test stand. Six such cells were contained in each pine box as shown in Figure 1. Five such boxes, set side by side constituted the holding facilities for these studies. After completion of the first generation study - first replicate - the exterior ends and partitions of the cages were painted white and cardboard sheets were placed between boxes to reduce visual contacts. At the same time 3/8" galvanized hardware cloth was fastened to each electrode to reduce the size of the electrode interstices between cells in a box.

3.4 Design and Treatments

3.4.1 - Three studies were conducted: (1) a first generation study with fetal examination for teratogenic effects, (2) a modified replicate first generation study without fetal examination for teratogenic effects, and (3) a second generation fertility study. The latter two were conducted simultaneously. All of the adults alive at weaning in the first study were examined histologically for morphological abnormalities.

3.4.2 - At the outset of each first generation study, the female and male rats were randomly assigned to five treatment groups as follows.

No. of Animals		
Male	Female	Treatment
		Study (1)
10	20	Control
10	20	45 Hz, 1.0 gauss, 10 v/m
10	20	45 Hz, 2.0 gauss, 20 v/m
10	20	75 Hz, 1.0 gauss, 10 v/m
10	20	75 Hz, 2.0 gauss, 20 v/m
		Study (2)
10	20	Control
10	20	45 Hz, 1.0 gauss, 20 v/m
10	20	45 Hz, 2.0 gauss, 20 v/m
10	20	75 Hz, 1.0 gauss, 20 v/m
10	20	75 Hz, 2.0 gauss, 20 v/m

3.4.3 - Ninety 45 day-old rats for the second generation study were selected from the original treatment groups and assigned to treatments as follows, with no brother-sister matings:

	No. of Animals		
Original Treatment	Male	Female	Final Treatment
Control	10	20	Control
45 Hz, 1.0 gauss, 10 v/m	0	3	45 Hz, 1.0 gauss, 20 v/m
45 Hz, 1.0 gauss, 10 v/m	0	4	45 Hz, 2.0 gauss, 20 v/m
45 Hz, 2.0 gauss, 20 v/m	5	7	45 Hz, 1.0 gauss, 20 v/m
45 Hz, 2.0 gauss, 20 v/m	5	6	45 Hz, 2.0 gauss, 20 v/m
75 Hz, 1.0 gauss, 10 v/m	0	3	75 Hz, 1.0 gauss, 20 v/m
75 Hz, 1.0 gauss, 10 v/m	0	4	75 Hz, 2.0 gauss, 20 v/m
75 Hz, 2.0 gauss, 20 v/m	5	7	75 Hz, 1.0 gauss, 20 v/m
75 Hz, 2.0 gauss, 20 v/m	5	6	75 Hz, 2.0 gauss, 20 v/m

It can be seen that considerable shifting of treatments within the 45 Hz and 75 Hz groups was carried out about three weeks after weaning. Reasons for this will be discussed in Section 5.0

3.4.4 - All rats commenced their respective (final) treatments at 45-70 days of age and continued exposures without interruption except for occasional equipment failure or daily 30-60 minute housekeeping activities.

3.4.5 - The 45 Hz, the 75 Hz, and the control treatments were carried out in three separate rooms.

3.5 Environmental Controls

Each of the three testing rooms was maintained at 76° ± 2 F during the study, except on a total of 10 scattered days over a period of nine months on which the 45 Hz room briefly went outside that range

to as high as 89° F and as low as 66° F. Each room had separate thermostatic control and temperatures were monitored twice daily. (Corrective measures were taken within two hours to restore temperature regulation on each occasion.) Ambient room lighting provided by overhead fluorescent lamps was equated by photometric measurement at the cage tops. A nine hour on-15 hour off light cycle was maintained. Airflow through the rooms exchanged the volume 12 times per hour with 100% fresh air. Resistance of the substrate in each cell was measured daily in situ with a volt-ohm meter and tap water was added at the top and lightly mixed into the top layer, as needed, to hold resistivity below 5000 meter-ohms. About every three weeks, the top half of the substrate was sifted to remove food and dried feces. The entire contents of each cell was exchanged for clean material prior to breeding in each study.

3.6 Procedure

3.6.1 - Sixty days prior to the start of mating, the 45-day old rats, having been laboratory acclimated for 15 days, were placed in the plastic boxes on the test fixtures for the first generation study - first replicate.

A similar procedure was followed in the second replicate except that the rats were 70 days old when put on the test stands.

The offspring of the first generation - first replicate - study, selected for the second generation study at their weaning, remained on the test stands until completion of the study, except for short periods needed to clean or replace the substrate.

3.6.2 - Breeding was accomplished in each instance by allowing one male to occupy a double cage with two females for a maximum of 21 days. Double cages resulted from removal of partitions overlying the even numbered electrodes in each pine box. Pregnant females were separated by placement in single cages.

3.6.3 - The day of mating was determined in the first generation study-first replicate-by daily microscopic examination of a vaginal saline wash. Presence of viable sperm indicated Day 0 of pregnancy. In the other studies, pregnancies were determined by abdominal palpation.

3.6.4 - On Day 14 of gestation, those females preselected for Caesarian sectioning (and deemed pregnant) in the first generation study - first replicate - were killed with chloroform and the uterine contents examined. Any grossly abnormal fetuses were preserved in 10% neutral buffered formalin for future reference.

3.6.5 - All other pregnant females were allowed to continue to term and to nurse or otherwise dispose of their litters ad libitum.

3.6.6 - Males were removed to separate cages on the test stands as soon as both assigned females were judged pregnant in the first generation - first replicate. In the other studies the males were sacrificed at that point.

3.6.7 - All continuing animals of both sexes remained in the EM fields until the last offspring were weaned. At that time all remaining adults were necropsied and representative tissues preserved in 10% neutral buffered formalin for histopathological examination (first generation study - first replicate - only).

3.6.8 - Water was available from water bottles in the plastic cages throughout the studies. Wayne Lab-Blox® rat food pellets were available in sufficient numbers to allow ad libitum eating without excessive buildup in the substrate.

3.7 Observations

3.7.1 - In the females on which Caesarian sections were performed, the following were observed and recorded:

- Number and placement of uterine implantation and resorption sites
- Number and placement of live and dead fetuses
- Total weight of fetuses (dead and alive), crown to rump length of fetuses, and any gross abnormalities of fetuses

3.7.2 - The following observations of the naturally delivered offspring in each litter were recorded:

- Number of live births and stillbirths by sex
- Number of deaths and of pups weaned
- Body weights at 24 hours, four days, and 21 days
- Any abnormalities of anatomy, behavior, or development

3.7.3 - Representative sections of the following tissues were stained in hematoxylin and eosin and microscopically examined for morphological abnormalities: brain (cerebrum, cerebellum, medulla), pituitary, thyroid, adrenal, heart, lung, spleen, liver, kidney, stomach, pancreas, small intestine, large intestine, testes/ovary, skeletal muscle, and peripheral nerve.

4.0 RESULTS

4.1 First Generation - First Replicate

A summary of the fertility, lactation, and weaning data is presented in Table C1. No meaningful differences were noted in mating behavior or conception rates between the control and test animals, and the fertility indices were generally comparable except for a slightly lower fertility index in the 45 Hz, 20-volt per meter group when compared with the control group. The gestation and live birth indices, as well as the duration of gestation, were comparable between control and treated animals.

A somewhat higher than normal mortality among the pups was observed during the lactation period in all groups including the controls. The lactation indices, though somewhat low among all groups, were generally comparable among control and treated groups. The mean body weights of the pups at 21 days were considerably lower than expected, but were comparable between control and treated groups.

Uterine and fetal data from Caesarean sections performed at 14 days of gestation are also presented in Table C1. There were no meaningful differences between the control and treated groups in the number and placement of implantation sites, resorption sites, and live fetuses and the appearances of the fetuses were not remarkable.

4.2 First Generation Study - Second (modified) Replicate

A summary of the fertility, gestation, lactation, and weaning data for this study is presented in Table C2. Again, there were no meaningful differences between the control and treated groups in any of the indices reported. The indices were likewise comparable to those of the first replicate of the first generation study in each respective group, except for a slightly lower fertility index recorded in all groups in this latter replicate.

4.3 Second Generation Study

A summary of the fertility, gestation, lactation, and weaning data for the second generation animals is presented in Table C3. There were no meaningful differences between control and treated groups in any of the indices except for a lower lactation index in the controls than in the combined EM-field groups. As in the first generation, the lactation indices were considerably lower than anticipated, as were the mean body weights of the pups at 21 days.

4.4 Combined Data

When the data of all three studies were combined, despite the differences in procedure among them, the control groups had a somewhat but not quite significantly higher fertility index than the pooled EM exposure groups ($\chi^2 = 2.74$, df = 1, $.10 < p > .05$). The lactation indices, in the same comparison, were very significantly different ($\chi^2 = 46.7$, df = 1, $p < .001$) with the combined control groups lower than the combined EM exposure groups. No other pooled data yielded a χ^2 with a probability of less than .40.

4.5 Histopathology

Table C4 presents the incidences of histopathology observed. Thyroid and ovarian activity were within normal limits for all animals and were essentially comparable between control and test groups. No alterations of any kind were observed in the small intestine, large intestine, skeletal muscle, or peripheral nerve in any animals. A number of expected spontaneous alterations were present in both control and exposed animals. They either occurred with relatively equal incidence and severity in control and exposed rats or occurred so rarely as to be considered unrelated to the EM conditions. No specific pathological alterations were detected which were considered effects of the EM fields.

5.0 DISCUSSION

No significant adverse effects of EM exposures were seen in these studies. The only significant effect was on the lactation index which was higher in the EM exposed groups than in the control groups combined. The meaning of this difference is unclear; it is difficult to visualize a mechanism whereby the EM exposure could increase the survivability of viable pups between 24 hours and 21 days postpartum. Closer examination of the data showed that a significant difference was obtained only in the second generation study and was thus not replicated throughout. No good explanation for this outcome in the second generation study can be suggested. In any case, the effect was not one deleterious to the species.

The different fertility indices for the pooled data approached statistical significance with the controls being higher than the EM exposed groups. The number of females mating and the time to observation of sperm were quite similar in all groups in the first replicate of the first generation study where these data were obtained. These data give no support to the notion that the EM fields might have interfered with mating behavior or female estrus

cycling. Since the results do not demand an explanation, further speculation on mechanisms is unwarranted.

The most striking outcome of these studies was the low lactation indices encountered throughout. The mean percentage of pups surviving from 24 hours to 21 days was 42.9%. This value is far below what was expected from preliminary work and from common rat breeding experience and thus requires some comment. The conditions under which the pups were reared were quite unusual as required by the nature of the experimental variables. The adult female rats were in close proximity, in visual auditory, and olfactory contact with at least one other adult during gestation and the perinatal period. Some tactual contact was also possible. In the first replication of the first generation study, it was found that some neonatal pups moved from cell to cell through the interstices in the electrodes as these became exposed by the adults digging in adjacent cells. The pups usually were destroyed by the adult males or were inadequately fostered by females, as the case may have been. In the later studies, this particular problem was rectified by the insertion of hardware cloth on one face of the electrodes. However, the contacts between cells were not eliminated by the measures taken to reduce visual contacts described in Section 3.3. Whether these contacts or some other factor(s) associated with the housing conditions were responsible for the low survival rate cannot be ascertained. The important point to be made, however, is that the EM conditions were not in any way involved.

Because of the small numbers of offspring available in some treatment groups to be used to breed the second generation, considerable shifting from one level to another within frequency groups was required to achieve a balanced design. Thus, five males and seven females in each frequency group were shifted from a 2.0 gauss to a 1.0 gauss environment about three weeks after weaning, and, similarly, seven females in each frequency group were shifted from a 10 v/m to a 20 v/m environment. No frequency interchanges were made. Although these shifts were not originally planned and did render the design less than elegant, they were not deemed to invalidate in any way the conclusion that 10-20 v/m, 1.0-2.0 gauss EM fields did not adversely affect reproductive parameters in the rat.

6.0 APPENDICES

6.1 Tables of Data

Table C1 - Summary table of data/indices for the First Generation Study - first replicate

MEASUREMENTS	CONTROL	TREATMENT 45 HZ 10 V/M 1 GAUSS	45 HZ 20 V/M 2 GAUSS	75 HZ 10 V/M 1 GAUSS	75 HZ 20 V/M 2 GAUSS
No. females paired with males	20	20	20	20	20
No. pregnancies	18	18	14	16	17
Fertility index	90%	90%	70%	80%	85%
No. females mating	20	19	18	20	18
Mean days from pairing to observation of sperm	5.45	3.89	5.06	6.10	4.94
FULL TERM DELIVERIES					
No. pups delivered	96	87	77	84	98
Mean litter size	10	11	11	10	10
No. pups alive at 24 hours	92	78	66	81	93
Live birth index	95.8%	89.7%	85.7%	96.4%	94.9%
Mean pup weights (grams):					
24 Hours - males	6	6	6	7	7
- females	6	6	6	6	7
4 Days - males	8	8	8	9	9
- females	8	8	8	9	8
21 Days - males	24	26	26	21	24
- females	27	25	25	20	21
No. pups weaned	40	32	19	41	57
Lactation index	43.5%	41.0%	28.8%	50.6%	61.3%
CAESARIAN DELIVERIES					
Mean no. of:					
Implantation sites	11.1	11.4	12.6	11.1	13.1
Resorption sites	2.0	1.4	1.6	0.4	1.3
Live fetuses	9.0	10.0	10.9	10.7	11.9
Dead fetuses	0.1	0	0.1	0	0
Mean fetal weight (grams)	0.14	0.13	0.14	0.15	0.15
Mean fetal length (cm)	1.3	1.3	1.3	1.3	1.3

Table C2 - Summary table of data/indices for the First Generation Study - second (modified) replicate

	TREATMENT				
		45 HZ		75 HZ	
		20 V/M	20 V/M	20 V/M	20 V/M
MEASUREMENT	CONTROL	1 GAUSS	2 GAUSS	1 GAUSS	2 GAUSS
No. females paired with males	20	20	20	20	20
No. pregnancies	15	10	15	13	12
Fertility index	75%	50%	75%	65%	60%
No. pups delivered	127	91	149	128	114
Mean litter size	8	9	10	10	10
No. pups alive at 24 hours	120	91	132	128	113
Live birth index	94.5%	100%	88.6%	100%	99.1%
Mean pup weights (grams):					
24 Hours - males	8	8	8	8	8
- females	7	8	7	7	8
4 Days - males	12	11	9	10	10
- females	9	10	10	9	10
21 Days - males	20	30	23	28	29
- females	23	27	23	27	25
No. pups weaned	37	60	64	48	61
Lactation index	34.6%	65.9%	49.2%	37.5%	54.0%

Table C3 - Summary table of data/indices for the Second Generation Study

	TREATMENT				
		45 HZ		75 HZ	
		20 V/M	20 V/M	20 V/M	20 V/M
MEASUREMENT	CONTROL	1 GAUSS	2 GAUSS	1 GAUSS	2 GAUSS
No. females paired with males	20	10	10	10	10
No. pregnancies	19	10	9	7	10
Fertility index	95%	100%	90%	70%	100%
No. pups delivered	229	108	101	78	110
Mean litter size	13	11	11	11	11
No. pups alive at 24 hours	229	102	96	76	110
Live birth index	100%	94.4%	95.0%	97.4%	100%
Mean pup weights (grams):					
24 Hours - males	7	8	7	7	7
- females	6	7	7	6	7
4 Days - males	9	10	9	10	9
- females	8	8	9	8	9
21 Days - males	19	17	19	31	26
- females	20	19	22	30	25
No. pups weaned	50	37	46	28	69
Lactation index	23.3%	36.3%	47.9%	36.8%	62.7%

Table C4 - Histopathology incidence table

ORGANS	MALES					FEMALES				
		45 HZ		75 HZ			45 HZ		75 HZ	
	CONTROL	10 V/M 1 GAUSS	20 V/M 2 GAUSS	10 V/M 1 GAUSS	20 V/M 2 GAUSS	CONTROL	10 V/M 1 GAUSS	20 V/M 2 GAUSS	10 V/M 1 GAUSS	20 V/M 2 GAUSS
Brain - No. examined	10	10	10	10	10	10	10	10	10	10
Lymphocytes/pineal	0/10	2/10	0/10	0/10	0/10	1/10	1/10	1/10	0/10	0/10
Pituitary - No. examined	9	7	7	5	9	9	9	8	9	9
Cysts	1/9	0/7	1/7	0/5	0/9	1/9	1/9	0/8	0/9	1/9
Adrenal - No. examined	10	9	10	10	9	10	10	10	10	10
Cortical nodular hypertrophy	0/10	0/9	0/10	0/10	0/9	1/10	0/10	0/10	0/10	0/10
Cortical vacuolation	0/10	0/9	1/10	0/10	1/9	0/10	0/10	1/10	0/10	0/10
Cystic degeneration	0/10	0/9	0/10	0/10	0/9	1/10	0/10	0/10	0/10	0/10
Heart - No. examined	10	10	10	10	10	10	10	10	10	10
Epicarditis	0/10	0/10	1/10	1/10	2/10	0/10	0/10	0/10	0/10	0/10
Focal myocarditis	3/10	2/10	2/10	3/10	4/10	0/10	2/10	1/10	1/10	0/10
Lung - No. examined	10	10	10	10	10	10	10	10	10	10
Interstitial pneumonitis	5/10	9/10	10/10	4/10	6/10	6/10	9/10	10/10	10/10	8/10
Lymphoid hyperplasia	10/10	9/10	10/10	9/10	8/10	9/10	10/10	10/10	10/10	10/10
Medial vascular hypertrophy	6/10	6/10	6/10	2/10	5/10	6/10	6/10	8/10	4/10	3/10
Spleen - No. examined	10	10	10	10	10	10	10	10	10	10
Pigment	0	0	1	0	1	1	0	2	3	1
Extramedullary hematopoiesis	4	1	2	1	0	0	0	1	0	1
Liver - No. examined	10	10	10	10	10	10	10	10	10	10
Hepatitis	0	0	0	0	1	1	0	0	0	1
Pericholangitis	5	6	4	6	5	6	9	9	6	5
Mononuclear infiltrate	2	5	3	5	3	2	1	2	2	2

Table C4 - Continued

ORGANS	MALES					FEMALES				
		45 HZ		75 HZ			45 HZ		75 HZ	
	CONTROL	10 V/M 1 GAUSS	20 V/M 2 GAUSS	10 V/M 1 GAUSS	20 V/M 2 GAUSS	CONTROL	10 V/M 1 GAUSS	20 V/M 2 GAUSS	10 V/M 1 GAUSS	20 V/M 2 GAUSS
Kidney - No. examined	10	10	10	10	10	10	10	10	10	10
Dilated pelvis	0	0	0	0	1	0	0	0	0	0
Interstitial nephritis	2	2	5	4	4	1	2	3	4	2
Mineral deposition	1	0	0	0	0	1	1	1	1	1
Regenerative epithelium	1	2	2	0	2	0	0	1	0	0
Pyelitis	0	0	0	0	0	0	0	0	1	0
Stomach - No. examined	10	9	10	10	9	10	10	9	10	10
Gastritis	0	0	0	0	0	0	1	0	0	0
Mineral deposition	0	0	0	0	0	0	0	0	0	1
Testis - No. examined	10	10	10	10	10					
Aspermatogenesis	0	0	0	1*	1*					
Hypospermatogenesis	0	0	0	1	0					
Uterus - No. examined						3	2	8	4	6
Distended lumen						2	1	4	2	1
Pigment deposition						1	1	4	3	5

* Unilateral

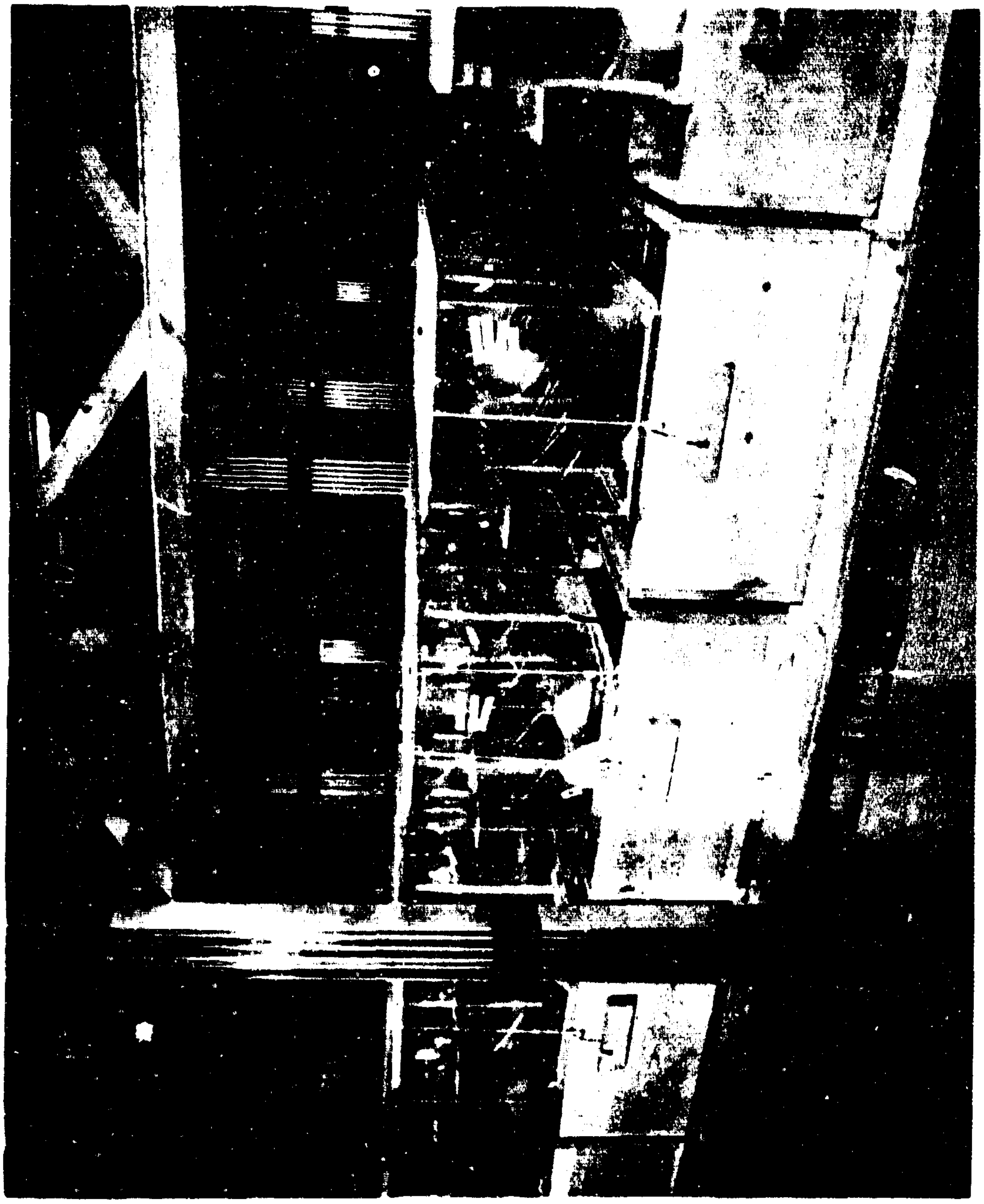


Figure C1 - Plastic rat cages shown in position over the E-field electrodes. Magnetic field coils are visible hanging from overhead racks.

CHAPTER D
CANINE PHYSIOLOGICAL STUDY

Winnie R. Peeters
William B. Coate

TABLE OF CONTENTS

1.0 INTRODUCTION

Chapter D contains the methodology and results of a canine physiological study performed in fulfilling the requirements of the Naval Electronics Systems Command, Contract No. NOOO-39-69-C-1572. The objective of this contract was to assess preliminarily the possibilities of biological/ecological impact from the continuous presence in a given locale of electric fields, magnetic fields, and earth return currents of specified magnitudes. The present study was performed as a pilot study to determine whether gross effects on certain physiological and clinical laboratory parameters could be expected in mammals from these levels of electromagnetic fields and/or earth return currents at extremely low frequency (ELF).

2.0 SUMMARY

In a canine physiological study pairs of beagle dogs were exposed overnight, five days per week, for three weeks to electromagnetic fields of 10 volts per meter and 1.0 gauss or 20 volts per meter and 2.0 gauss at either 45 or 75 Hz while receiving 0.5 mA body current at the respective frequencies through leg electrodes or to control conditions.

No evidence of an effect of the electromagnetic input was noted on gross behavior, body weight, electroencephalogram, electrocardiogram, spermatozoa, ophthalmic structures, hematology, biochemistry, urinalysis, and gross appearance of tissues and organs at necropsy.

Two of eight exposed dogs had slightly elevated rectal temperatures during the exposure period. Seven exposed dogs had one or more elevated (>21 mm Hg) systolic and/or diastolic blood pressure value during exposure and/or postexposure periods, as did one of two control dogs during this period.

3.0 METHODS

3.1 Introduction

3.1.1 - In general, this study in dogs was based upon a medical follow-up study of high voltage lineman reported by Kouwenhoven, et al.(1).

3.1.2 - In the present study several physiological and clinical laboratory parameters were selected for evaluation on the basis that they would reflect the activity of the major body systems.

3.2 Animals

3.2.1 - Seven young adult male and seven female beagle dogs were used in this study. They were obtained from our own breeding colony and had been maintained under laboratory conditions for several weeks. They had been vaccinated for canine distemper, infectious canine hepatitis, and rabies and had passed a routine physical examination and clinical laboratory screen.

3.2.2 - The 12 animals were surgically prepared and trained for the special conditions of the study; one male and one female were maintained under routine housing conditions and were subjected only to blood, urine, and semen (male) sampling procedures; ophthalmic examination; and body weight and rectal temperature measurement. Ten of the surgically prepared animals were divided into five treatment groups, each containing one male and one female. The extra pair of surgically prepared dogs was subjected to all sampling, recording, and examination procedures but not to any treatment conditions, other than two weeks of training in the treatment rooms.

3.3 Apparatus

3.3.1 - Details of the simulation apparatus used to provide the electromagnetic fields and body current are contained in Chapter B of this report.

3.3.2 - During exposure to the electromagnetic fields, two dogs were restrained in an area approximately 6100 square cm provided by dividing a 50.0 x 122 cm rectangle crosswise into two halves and fencing in the area with wooden slats. The dogs were placed in these enclosures in tandem with their heads facing out opposite canvas-enclosed ends, see Figures D1 and D2, and their rear quarters in apposition in the center of the area. The canvas end had a bound circular hole in the center. A collar was slipped through the tubular binding and fitted to the dog's neck. Horizontal edges of the canvas were rigidly attached to a wooden frame but the vertical edges were fashioned into a sleeve which slipped vertically on a post of the end frame. Excess material in this plane permitted the dog to stand or

lie at will. Each dog was also fitted with a harness which was secured by loose ropes to the sides of the enclosure and to the horizontal bar over the dog's head. Collars and harnesses were fabricated of leather or cotton webbing with aluminum fastenings.

3.3.3 - Two electrodes, fabricated from fine mesh stainless steel screen into rectangles approximately 20 x 25 mm were positioned contralaterally on a fore- and hindlimb with the use of electrode paste (The Burdick Corporation) and secured in position by wrapping the limb with micropore tape (3M Company). Leads from the electrodes were fastened to a band of the tape which encircled the trunk. The leads were supported overhead from an elastic band attached to a rope which ran the length of the box from one head frame to the other. Current was passed through the dog's body with these electrodes while the animal was in the electromagnetic fields.

3.3.4 - An electric field for each pair of dogs was provided by two 50 cm x 50 cm sheets of aluminum foil, backed by cardboard, each hung just beyond the nose of each dog with the bottom of the electrode 5.0 cm above the surface on which the dogs stood.

The alternating magnetic field was developed from a set of four current carrying coils surrounding the table on which the dog stood. One coil is visible in Figure D1.

3.4 Surgical Procedures

3.4.1 - In a two-stage surgical procedure six males and six females were each prepared with a carotid artery loop for direct measurement of arterial blood pressure and implanted with five electrodes in the calvarium for obtaining electroencephalograms. Routine systemic antibiotic treatment was administered for a few days prior to and following surgery and local treatment was administered as necessary thereafter. Sterile surgery was performed under pentobarbital sodium anesthesia (30 mg/kg).

3.4.2 - A carotid loop was formed in the first operative stage by isolating a section of the common carotid artery and bringing it completely outside of the ventral cervical musculature and encasing the vessel in a sleeve of skin to form a loop. This preparation allows one to obtain a permanent record of directly obtained systolic and diastolic blood pressure at repeated intervals over an extended period of time with very little discomfort to the animal.

3.4.3 - Five silver electrodes were implanted in the calvarium in the second operative stage. Small spherical (ball) electrodes were formed by heating the end of silver wire. They were cleaned in acid and soldered to stranded stainless steel lead wire encased in biologically inert plastic. The other end of the lead wire was soldered to a miniature contact. Contacts for five ball electrodes and one silver ring electrode, which served as a ground, were potted in an appropriated configuration with dental acrylic (Lang Dental Manufacturing Co.) in a plastic strip connector. Small burr holes were drilled only through the outer bony shelf of the calvarium after freeing the area of muscle and connective tissue. The holes were dried with an air stream; a ball electrode was placed therein and affixed to the skull with dental acrylic. Bilateral frontal electrodes were placed 1 cm anterior to the coronal suture and 1½ cm lateral to the saggital suture. Bilateral occipital electrodes were placed 1 cm lateral to the saggital suture and 2 cm posterior to the coronal suture. The vertex electrode was placed 1 cm posterior to the coronal suture on the midline. The ground electrode was sutured to muscle over the occipital crest. Two stainless steel screws were placed equidistant laterally from the midline just anterior to the vertex electrode and were used as anchors for the mound of dental acrylic fastening the electrode connector to the skull.

3.4.4 - Approximately one week after implanting the electrodes, a piece of dacron velour was glued to the connector mound to serve as a medium for cell infiltration to narrow the dermal defect imposed by the exiting connector.

3.4.5 - Surgery was completed six weeks prior to initiation of training in the exposure rooms.

3.5 Design

One male and one female dog was assigned to each of five treatments, including a control. All dogs exposed to EM fields and body current were tested in a before, during, and after exposure sequence. The control dogs were tested in a parallel fashion. Thus, each exposed dog was its own control as well as having a nonexposed control. Another sex-pair of surgically prepared dogs was kept in their usual cages throughout the study to provide a control for the stresses imposed by restraint during the above sequence. A final sex-pair of nonsurgical dogs was cage-housed throughout to provide a control for surgery and sampling procedure effects.

3.6 Treatments

Two electrical frequencies were tested - 45 Hz and 75 Hz - each at two electric field strengths - 10 volts per meter and 20 volts per meter - and at two respective magnetic field strengths - 1.0 gauss and 2.0 gauss - concurrently. All dogs exposed to EM fields were also exposed to 0.5 mA body current at the respective frequencies. A control treatment, in which only the earth's magnetic field (approximately 0.6 gauss) and the ambient 60 Hz electric field (approximately 0.05 millivolt per meter) were present was used. The 45 Hz treatments were tested in one room, the 75 Hz treatments in another, and the control in a third. Treatments were applied for 16 hours overnight, five days per week for three weeks.

3.7 Environmental Controls

Each of the three testing rooms was maintained at 76° ± 2 F during the study except for five scattered days in which the temperature in the 45 Hz room exceeded this range for short periods. Each room had separate thermostatic control and temperatures were monitored twice daily. Ambient room lighting was equated by photometric measurement. A 9.0 hour on-15 hour off light cycle was maintained. Airflow through the rooms exchanged the volume 12 times per hour with 100% fresh air.

3.8 Training

3.8.1 - The dogs were trained to sit quietly with the neck flexed dorsally under light restraint so that blood pressure could be obtained from the carotid loop and to lie in a right lateral recumbent position for recording electrocardiograms. The animals were also trained to the restraint imposed by the treatment enclosure. This training program was initiated during surgical convalescence.

3.8.2 - After the dogs had accepted restraint in the enclosures for extended daytime periods, they were placed there for a few overnight stays. Then for two weeks just prior to initiating field exposure, each of the dogs was assigned a position in one of the treatment rooms and spent each night there for five consecutive nights a week. In the 45 and 75 Hz rooms the restraint enclosures were placed outside the measurable magnetic field and no electric field or current was present.

3.9 Procedure

3.9.1 - During the two-week training period in the exposure rooms, 12 dogs were placed in the restraint enclosures between four to five o'clock in the afternoon and removed to their usual cages the next morning between eight to nine o'clock. This procedure was followed for five consecutive nights with the dogs spending the other two nights in their cages. On days when the animals were to go into the treatment room for the night, food was removed from their cage at noon, but water remained available. Food and water were not available in the restraint enclosures.

3.9.2 - Urine samples were collected weekly in metabolism cages during the morning between the fourth and fifth nights in the exposure rooms. Animals from which samples were not obtained in this manner were catheterized for immediate urine removal. Ophthalmic examinations were conducted in the afternoons following urine collections.

3.9.3 - Venous blood was drawn weekly while the animals were in a fasted condition the morning after the fifth consecutive night in the experimental rooms. Body weights and rectal temperatures were recorded immediately after blood was drawn. Semen samples were collected from the males just before noon on this day. Physiological parameters were recorded in the following sequence from each dog on this day: electroencephalogram, electrocardiogram, and blood pressure.

3.9.4 - This same schedule was followed for the two dogs not subjected to surgery with the exception that electroencephalograms and blood pressure were not recorded.

3.9.5 - After the two weeks of training in the exposure rooms had been completed and two values or records had been obtained for all of the parameters, 10 of the 12 surgically prepared dogs were selected for the five treatment groups. Selection was based on consistency of the control values and records and tractability of the animals for the required procedures.

3.9.6 - Since to obtain the physiological measurements and records on 12 dogs required most of the day, assignment of the animals to treatment groups was based on the time of day the control values and records had been obtained. In so far as possible, each treatment group was assigned one male or female from which the parameters were recorded

early after removal from the exposure room and one of the opposite sex whose records were obtained late after removal, so that total elapsed hours from removal from treatment environment to record taking were consistent among the groups.

3.9.7 - The same schedule of treatment and measurement established during the two-week training period was maintained during a three-week exposure and two-week postexposure period. During the postexposure period, the restraint enclosures in the 45 and 75 Hz rooms were again positioned outside the magnetic fields, as done in the training period.

3.9.8 - During the training period, electroencephalograms, electrocardiographs, and blood pressure records were taken more often than once a week, since stabilization of these parameters is critically affected by training. Two control records were selected from any of those obtained during this period which coincided with the timing schedule maintained on dogs following the fifth consecutive night spent in the treatment rooms. Once treatments had been initiated, these parameters, as the others, were recorded only once a week. The eye examination, and all sampling, measurement, and recording procedures were conducted approximately the same time of day throughout the study for individual dogs to avoid any possible changes due to diurnal fluctuations in the parameters.

3.9.9 - Treatment assignment for the dogs was as follows:

Dog No.	Sex	Treatment
14786	♂	Control room - restrained
14680	♀	Control room - restrained
14800	♂	45 Hz - 1 gauss - 10 v/m
14819	♀	45 Hz - 1 gauss - 10 v/m
14797	♂	45 Hz - 2 gauss - 20 v/m
14818	♀	45 Hz - 2 gauss - 20 v/m
14798	♂	75 Hz - 1 gauss - 10 v/m
14739	♀	75 Hz - 1 gauss - 10 v/m
14783	♂	75 Hz - 2 gauss - 20 v/m
14836	♀	75 Hz - 2 gauss - 20 v/m
14799	♂	Control - conventional cage
14825	♀	Control - conventional cage
14790	♂	Control - not surgically prepared, caged
14816	♀	Control - not surgically prepared, caged

3.9.10 - Within one week after termination of the study, the first 10 dogs above were sacrificed and representative tissues were taken and fixed for possible future histological examination.

3.10 Observations

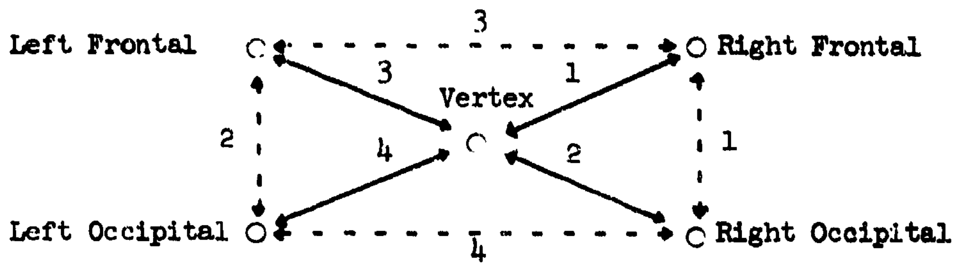
3.10.1 - Blood and blood serum biochemical determinations included sodium, potassium, chloride, carbon dioxide, serum albumin, total protein, albumin/globin ratio, serum electrophoresis, blood urea nitrogen, glucose, alkaline phosphatase, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, total lactic dehydrogenase and isozymes I and V, pO_2, pCO_2, and pH.

3.10.2 - Hematological determinations included hematocrit, hemoglobin, reticulocyte count, erythrocyte count, leukocyte count, and leukocyte differential count.

3.10.3 - Urinalysis included pH, specific gravity, sugar, protein, bilirubin, urobilinogen, appearance, microscopic examination of sediment, myoglobins, and 17-hydroxycorticosteroids.

3.10.4 - The electrocardiogram was recorded with a Sanborn Model 1500 A electrocardiograph at the standard paper speed of 25 mm/sec and calibration of 1 mv/cm. The dogs were restrained in a right lateral recumbent position. Ten leads were recorded; they were I, II, III, aVR, aVL, aVF, CV_6LL, CV_6LU, CV_5RL, and V_{10}. The first six leads are standard and are the same as used in human cardiology; the last four are special chest leads for the dog and have been described by Detweiler, et al.(2).

3.10.5 - Four-channel electroencephalograms were recorded in two modes with a Grass Model 7 polygraph using Model 7P5 wide band ac preamplifiers. Paper speed was 25 mm/sec and calibration was 50 µV/cm with the recording being made at a sensitivity setting of 75 µV/cm. Mode A paired left and right frontal and occipital electrodes with the vertex electrode and Mode B paired frontal and occipital electrodes bilaterally and hemispherically. A diagramatic depiction and listing of these modes follows.



Mode A

Channel 1 - Right frontal - Vertex
2 - Right occipital - Vertex
3 - Left frontal - Vertex
4 - Left frontal - Vertex

Mode B

Channel 1 - Right frontal - Right occipital
2 - Left frontal - Left Occipital
3 - Right frontal - Left frontal
4 - Right occipital - Left occipital

The dogs were restrained by the collar in an enclosure similar to that used during treatment and maintained in an alert condition while the electroencephalogram was recorded.

3.10.6 - Arterial blood pressure was obtained by introduction into the carotid artery loop of a hypodermic needle connected by an anticoagulant fluid system (heparin-saline) to a Sanborn Model 267B pressure transducer and recorded with a Sanborn Model 964 polygraph.

3.10.7 - Electrocardiograms, electroencephalograms, and blood pressure records were obtained with one animal alone in a quiet room with the same handlers on each occasion.

3.10.8 - The ophthalmic examination was conducted in a darkened room. One drop of 0.5% Mydriacyl® had been administered to the eyes of each dog at least 15 minutes prior to the examination. The anterior segment of the eye was first examined while directly illuminating the eye with a light beam. The fundus was examined with a binocular indirect ophthalmoscope.

3.10.9 - The dogs were observed daily, five days per week, for gross appearance and behavior and any abnormalities were recorded.

3.10.10 - Body weights and rectal temperatures were measured on a Toledo platform scale and with a clinical rectal thermometer, respectively.

3.10.11 - The animals were necropsied immediately following sacrifice and all major organs and tissues were observed for gross abnormalities.

4.0 RESULTS

4.1 Gross Observation

No changes were noted in gross behavior that were related to exposure in the electrical and magnetic fields. During the second exposure week, all dogs placed in the restraining enclosures, including the pair in the control room, appeared nervous when handled during placement or removal from the enclosure. Behavior appeared normal while the dogs were in their routine cages.

4.2 Body Weight

Any stress which may have been induced by field exposure was not reflected in adverse weight changes in the exposed dogs. Their body weight changes during the three weeks of exposure were minimal (-0.2 to +0.4 kg) and were similar to those changes incurred during this period by the various types of control dogs (-0.2 to +0.7 kg).

4.3 Rectal Temperature

Rectal temperature was variable from dog to dog and also from period to period within a single animal. Recorded values ranged from 100.4 to 104.3° F, with most of the values being 101 to 102° F. Male Dog No. 14783 (75 Hz, 20 v/m, 2 gauss) and male Dog No. 14797 (45 Hz, 20 v/m, 2 gauss) consistently had temperatures during the exposure period that were higher than their respective values during control and postexposure periods. All data for this parameter are presented in Table D1.

4.4 Electroencephalogram

No apparent changes were noted from visual analysis of the electroencephalograms of exposed dogs which were related to their treatment under the conditions of this study. Most of the dogs, controls as well as exposed animals, showed

increased synchronization, especially in leads from frontal areas, as the study progressed. This tendency was the only consistent change noted.

4.5 Electrocardiogram

Exposure in the electric and magnetic fields under the condition of this study did not appear to induce any electrocardiographic changes as determined by visual analysis of the records. No notable changes in sequence or rhythm of the electrical events of the heart were seen other than obvious changes in rate. There were some random changes in the character of the waves, such as small or absent P waves in some leads, inversion and change in magnitude of T waves, and occasional low voltage in several leads. None of these changes were consistent nor considered grave modifications. These changes were not considered to be related to field exposure due to their random occurrence and appearance in control as well as exposed dogs. They are commonly observed in repeated measurements due to slight shifts in positions of the heart relative to the limbs.

4.6 Blood Pressure

4.6.1 - The two control blood pressure values for each dog were summed and individual means determined. Subsequent systolic and/or diastolic values which differed from these means by 21 or more mm Hg were considered significant changes. All blood pressure values, including the change from mean control, are presented in Table D2.

4.6.2 - The male dog maintained in its cage after the training period exhibited one significantly depressed blood pressure value during the weeks corresponding to the exposure and postexposure periods. Both dogs in the control room had significant falls in pressure also and the male had one significantly elevated value. Two considerably different blood pressure values (130/85 and 185/110 mm Hg) were recorded for this male the fifth week (corresponding to the third exposure week); the systolic pressure of the second value was significantly greater (+43 mm Hg) than his control mean. Each tracing for this animal for this particular period was equally acceptable since both were relatively uniform throughout the entire record. Both values are listed since there was no rational basis for discarding either.

4.6.3 - All significant changes in blood pressure for exposed animals were elevations of pressure. Three of the four dogs

exposed to 45 Hz and all four of those exposed to 75 Hz exhibited an elevated pressure one or more times during the exposure or postexposure period. The female exposed to 45 Hz at 10 v/m, 1 gauss and 0.5 mA, had a significantly elevated pressure only after the third exposure week. The male exposed to the higher field strengths of this frequency consistently had elevated blood pressure during the exposure and postexposure period and the female was similarly affected after the first and third weeks of exposure. The male exposed to 75 Hz at 10 v/m, 1 gauss had an elevated pressure the third exposure week and again the last week of the study; the similarly treated female exhibited an elevated pressure only the first postexposure week. At the higher field strengths of this frequency, the male's blood pressure was elevated the third week of exposure and the female's pressure was higher the first and third weeks of exposure and the first week postexposure.

4.7 Sperm Count and Microscopic Examination

4.7.1 - There did not appear to be any effects on the number of spermatozoa or their morphology and motility which were related to EM exposure. The sperm count was quite variable from dog to dog and also from sample to sample for some of the dogs.

4.7.2 - Samples from the control male maintained in his cage throughout the exposure and postexposure periods were not of sufficient volume for count and examination. Counts for the sampling control were essentially consistent except for a decreased count for the sample obtained the last week of the study; however, spermatozoa in the fourth sample (corresponding to the second exposure week) were nonmotile and approximately 60% exhibited coiled tails. Movement and percentage motility improved with subsequent samples. The control room male had increased counts (compared with his previous counts) during the weeks corresponding to the exposure period. Likewise, increased counts were recorded for one to two weeks of this period for males exposed to 45 Hz. The count was consistent throughout the study for samples from the male exposed to the low level 75 Hz treatment. For the male exposed to the high level of this frequency, counts were consistently higher during and after treatment. For all the animals, other than the one mentioned above, sperm morphology was essentially normal and movement and percentage of motile cells were directly related to the sample cell population - sperm from samples with higher counts were more active and there were relatively fewer nonmotile cells.

4.8 Ophthalmic Examination

Changes noted in the eyes of the dogs in this study did not appear to be correlated with electromagnetic exposure. In the control room male and one exposed male (75 Hz, 10 v/m, 1 gauss) a small corneal lesion or opaque spot was noted in one eye only in one examination during the control period and not again, but in a third male (45 Hz, 10 v/m, 1 gauss) a similar finding noted in the control period persisted throughout the study. Slight conjunctivitis was noted during one examination of the male sampling control. In females in 20 v/m, 2 gauss fields of both frequencies, findings noted initially persisted with slight changes throughout the study. Additionally, the female treated at the lower frequency showed bilateral conjunctivitis during the postexposure period and the female at the higher frequency had a hemorrhagic area over the fundus after the first exposure week and a pannus on the limbus the second week; these conditions persisted throughout the remainder of the study.

4.9 Hematology

None of the hematological changes noted during this study appeared to be related to field exposure. Values from the dogs in this study were compared with historical values cumulated at Hazleton Laboratories from 215 to 542 male and female beagles. Several scattered hematological values were outside the historical normal range. However, the abnormal values were just outside the normal limits in most instances, occurred during the control period for some animals, and were found in control as well as exposed animals. No significance was attached to these randomly occurring "abnormal" values.

4.10 Clinical Biochemistry

4.10.1 - The treatments did not appear to induce adverse changes in determined biochemical parameters. The results for many of the parameters were compared with historical normal ranges cumulated from 512 to 533 male and female beagles studied at our laboratory; these parameters were the more common ones usually studied in toxicologic evaluation and samples were analysed using Technicon's SMA-12 Autoanalyzer. Parameters for which no historical normal ranges were available were evaluated by comparing subsequent values with those obtained in the control period.

4.10.2 - The statement made concerning the occurrence of scattered abnormal hematological values applies equally well to the biochemical results in this study. Since no pattern could be distinguished in the random occurrence of the "abnormal" values, no significance was attached thereto. One notable biochemical finding was a marked elevation of transaminase activity (both oxaloacetic[193] and pyruvate[125 plus]) after the first postexposure week for female Dog No. 14836, exposed to 75 Hz at 20 v/m, 2 gauss. One week later (last week of the study) both transaminase activities were still slightly elevated (58 and 49, respectively).

4.10.3 - Several dogs, controls as well as exposed animals, had slightly elevated serum glutamic oxaloacetic transaminase values, particularly during the postexposure period. Slightly elevated serum glutamic pyruvate transaminase values were also obtained initially and during the postexposure period for a control dog, initially for an exposed dog and after the third week of treatment for another exposed dog.

4.11 Urinalyses

No consistent abnormalities were noted in urine samples from control or exposed dogs. Microscopic examination showed a higher prevalence of cellular constituents (erythrocytes, leukocytes, and epithelial cells) in samples from the females, but this is to be expected due to vaginal contamination. Increased protein occurred on one occasion during the exposure period from Dog No. 14800 (45 Hz, 10 v/m, 1 gauss) and in samples from the male and female sampling controls; the same sample from the exposed dog also contained occult blood.

4.12 Necropsy

4.12.1 - Of the eight exposed and two control room dogs necropsied at termination of the study, only four had notable gross findings and these were not considered related to exposure in the electric and magnetic fields.

4.12.2 - The small intestine of the control room male appeared reddened and congested. The urinary bladder of the female exposed to 45 Hz at 10 v/m, 1 gauss appeared slightly irritated; a similar condition was noted for the male in this field, along with a reddened and irritated small intestine and a darkened area extending along the greater curvature of the spleen. The male exposed to 75 Hz at 10 v/m, 1 gauss also had a darkened area on the large end of the spleen.

5.0 DISCUSSION

Of the several parameters evaluated in this canine physiological study, there was an indication of possible effects induced by the electromagnetic input only on rectal temperature and blood pressure.

Evidence for there being a real effect of the treatments on body temperature is meager since only two of eight exposed dogs appeared to have slightly elevated rectal temperatures during the exposure period. Moreover the temperatures of the two dogs were not consistently as high during this period as occasionally recorded for the other dogs. The basis in evidence for a possible effect lies not as much on the magnitude of the elevation as on the consistency of the change and its coincidence with exposure only. The heating effect of very and super high frequency electromagnetic radiation is well-known, but the threshold for this effect is at least one order of magnitude greater than the strength of the electrical field of the present study.

Evidence for induction of a hypertensive effect from the EM treatments is much stronger than that for an effect on body temperatures since seven of the eight exposed animals showed an elevation in blood pressure at one time or another during exposure or in the two weeks immediately after treatment. The finding of an elevated pressure in one control dog during this period, however, does tend to weaken the evidence although this dog's pressure was apparently normal immediately prior to obtaining the elevated value.

It is obvious that the male in the control room had the highest control pressure of any of the dogs placed in the restraining enclosures and, for this reason alone, one might expect the level to subsequently decrease (which it did) with further training if this were not the true baseline pressure. At the time of assignment of the dogs to treatments, each systolic value available for this animal had been consistently higher than the usual level seen in most beagles and it had to be assumed that this slightly higher systolic level was normal for this animal.

It is considered noteworthy that five of the seven dogs showing the hypertensive effect had an elevation of diastolic pressure. The systolic pressure is subject to wider variations under ordinary conditions of health than the diastolic; for instance, the systolic is markedly altered by emotional influences. It also varies more with local arterial changes.

In considering the evidence for an effect of the EM treatments on blood pressure, one must be cognizant of the extremely labile nature of this parameter. The two markedly different values obtained within a short time span for the control room male provides a striking example of this characteristic. It is also pertinent to state that because of the labile nature of the blood pressure there usually are fluctuations in the records and consequently there is a certain amount of subjectivity in judging the overall baseline pressure in a particular record. The method used in the present study whereby a continuous tracing of several minutes' duration can be obtained affords a marked advantage in judging the baseline over methods utilizing indirect measurement of pressure wherein only periodic values can be obtained.

The marked elevation in transaminase activity in Dog No. 14836 is puzzling; no indication of the contributing cause was found. No evidence of cardiac infarction could be distinguished by careful study of the electrocardiogram obtained on the same date nor was there any evidence of hepatic injury noted in biochemical results from that day.

On Thursday, June 4, during the second exposure week, three of the dogs in the 75 Hz room received sufficient current, apparently from a lightning bolt which struck the feeder power line to the building (and burned out a transformer in the air-conditioner), to partially disintegrate the electrodes secured to the legs and to char the skin immediately thereunder. The electrical storm occurred after normal working hours and the condition of the dogs was not discovered until the following morning. Female Dog No. 14836, also in this room, did not have burns on her legs; one of the electrode lead wires was found broken the next morning and this is the probable explanation for the lack of burns.

No apparent lasting effects, other than local burns, resulted from this momentary surge of current through the animals. The lesions healed without any complications.

Each exposed dog did not receive electrical current through the leg electrodes throughout the entire night for each of the 15 nights of exposure since on several occasions some of the electrodes were found outside the taped area the next morning or the lead wire had broken. The minimum of nights with current was 10 (Dogs No. 14739 and No. 14798) and the maximum was 14 (Dog No. 14818).

6.0 APPENDICES

6.1 Tables of Data and Figures

Table D1 - Rectal temperature (° F) for male and female beagle dogs exposed overnight, five days per week, for three weeks to various electromagnetic fields while receiving 0.5 mA body current or to control conditions

	TREATMENT													
	45 HZ				75 HZ									
	10 V/M 1 GAUSS		20 V/M 2 GAUSS		10 V/M 1 GAUSS		20 V/M 2 GAUSS		CONTROL ROOM		CAGE CONTROL		SAMPLING CONTROL	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
WEEKS	14800	14819	14797	14818	14798	14739	14783	14836	14786	14680	14799	14825	14790	14816
Pre-exposure	101.2	104.0	101.6	101.8	102.4	101.2	101.4	101.0	101.6	100.6	103.0	101.2	101.6	101.8
Pre-exposure	101.4	102.0	101.5	101.5	101.7	101.4	101.7	101.7	103.0	100.8	101.5	101.6	-	101.5
Exposure	101.4	101.2	103.0	101.6	102.4	101.6	103.0	101.0	101.2	101.1	101.4	100.4	100.6	101.4
Exposure	102.0	103.0	104.3	101.8	102.2	102.0	102.6	102.2	101.6	102.4	102.0	101.4	102.0	102.4
Exposure	101.0	102.4	102.2	102.0	101.6	102.0	102.2	102.0	101.2	101.4	102.0	102.6	101.0	102.2
Postexposure	101.2	101.8	101.4	101.8	101.2	101.4	100.2	101.2	101.4	103.2	102.0	102.2	100.6	101.4
Postexposure	101.4	102.2	101.4	102.0	102.4	101.4	101.8	101.4	101.8	101.0	101.4	101.8	101.6	102.0

Table D2 - Blood pressure and changes from control (mm Hg) for male and female beagle dogs exposed overnight, five days per week, for three weeks to various electromagnetic fields while receiving 0.5 mA body current or to control conditions

	TREATMENT											
	45 HZ				75 HZ							
	10 V/M 1 GAUSS		20 V/M 2 GAUSS		10 V/M 1 GAUSS		20 V/M 2 GAUSS		CONTROL ROOM		CAGE CONTROL	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
WEEKS	14800	14819	14797	14818	14798	14739	14783	14836	14786	14680	14799	14825
A - SYSTOLIC/DIASTOLIC VALUES												
Pre-exposure	110/95	120/80	125/85	110/80	105/90	110/70	105/85	125/85	145/100	120/78	135/95	135/105
Pre-exposure	120/85	110/70	120/70	120/90	110/75	120/70	110/75	115/80	138/85	125/75	135/100	145/100
Exposure	110/80	105/80	140/100	150/100	115/80	115/85	105/80	145/100	130/100	115/80	100/80	120/85
Exposure	125/90	100/70	185/130	110/70	115/80	135/75	110/75	130/80	160/115	100/60	130/90	125/85
Exposure	130/85	200/135	130/100	140/95	135/90	135/85	130/115	140/115	130/85	110/65	130/85	125/85
									185/110			
Postexposure	115/90	130/90	150/115	120/75	120/90	170/120	125/90	140/110	140/90	140/85	135/95	130/95
Postexposure	115/85	130/95	175/130	125/100	130/95	130/85	120/90	125/85	120/85	125/85	135/90	125/90
B - SYSTOLIC/DIASTOLIC CHANGES FROM MEAN PRE-EXPOSURE VALUES												
Exposure	-5/-10	-10/-5	+17/+22	+35/+15	+7/+3	0/+15	-3/0	+25/+17	-12/-7	-8/+3	-35/-18	-20/-18
Exposure	+10/0	-15/-5	+62/+52	-5/-15	+7/+3	+20/+5	+2/+5	+10/-3	-18/-22	-23/-17	-5/-8	-15/-18
Exposure	+15/-5	+85/+60	+7/+22	+25/+10	+27/+7	+20/+15	+22/+35	+20/+32	-12/-8	-13/-12	-5/-13	-15/-18
									+43/+17			
Postexposure	0/0	+15/+15	+27/+37	+5/-10	+12/+7	+55/+50	+17/+10	+20/+27	-2/-3	+17/+7	0/-3	-10/-8
Postexposure	0/-5	+15/+20	+52/+52	+10/+15	+22/+12	+15/+15	+12/+10	+5/+2	-22/-8	+2/+8	0/-8	-15/-13

Figure D1 - General view of the dog restraining units in place for exposure to ELF electromagnetic fields

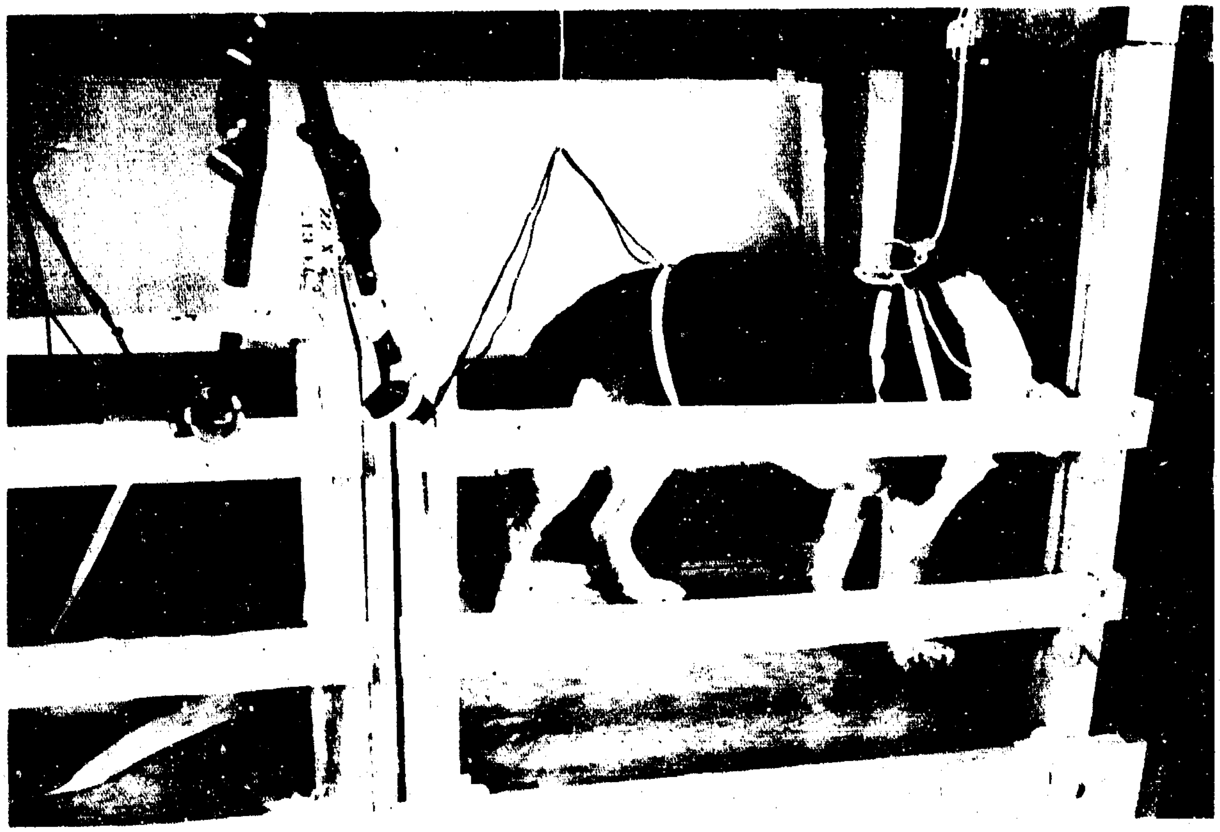


Figure D2 - Close view of dog in restraint with electrode wires showing

6.2 References

1. Kouwenhoven, W. B., Langworthy, O. R., Singewald, M. L., and Knickerbocker, G. G., Medical Evaluation of Man Working in AC Electric Fields, <u>IEEE Transactions on Power Apparatus and Systems</u>, Vol. PAS-86, pp. 506-511, April, 1967.

2. Detweiler, D. K., Melbin, J., and Hill, J. D., "Interpretation of Drug Induced Electrocardiographic Changes in Dogs in Evaluating Agents for Use in Man" in: <u>Importance of Fundamental Principles in Drug Evaluation</u>, edited by D. H. Tedeschi and R. E. Tedeschi, New York: Raven Press, pp. 422-424, 1968.

CHAPTER E
INSECT MUTAGENESIS STUDY

William B. Coate
William H. Negherbon

TABLE OF CONTENTS

1.0 INTRODUCTION

Chapter E contains the methodology and results of a mutagenesis study employing fruit flies (Drosophila melanogaster) performed in fulfilling the requirements of the Naval Electronic Systems Command, Contract No. N000-39-69-C-1572. The objective of this contract was to assess preliminarily the possibilities of biological/ecological impact from the continuous presence in a given locale of electric fields, magnetic fields, and earth return currents of specified magnitudes. The present study was performed as a pilot study to determine whether a 48-hour exposure to these levels of electromagnetic (EM) fields at extremely low frequencies (ELF) would induce a lethal or semilethal mutation on the X chromosome of male Oregon-R (wild-type) fruit flies.

2.0 SUMMARY

Oregon-R (wild-type) adult male Drosophila melanogaster were exposed to 45 Hz or 75 Hz EM fields or control conditions for 48 hours immediately prior to mating with virgin Muller-5 female Drosophila. Individually cultured F_1 heterozygous females x F_1 Muller-5 male crosses (P_2) were evaluated for F_2 female/male ratios and for Muller-5/Oregon-R male ratios. Successful F_2 cultures lacking Oregon-R males were taken as indicative of a recessive lethal X chromosome. F_2 cultures in which the number of Oregon-R males was less than one-half the number of Muller-5 males was taken as indicative of a "semilethal" X chromosome. Three hundred control and 300 exposed X chromosomes were thus examined. No lethal and 12 semilethals were found in the controls; 15 cultures without wild-type males and seven semilethals were found in the combined exposed F_2 cultures. Of the 15 "lethals," 14 lacked heterozygous females and were thus considered to have originated from Muller-5 females accidentally crossed with Muller-5 males in P_1 or as indicative of a delayed dominant lethal mutation.

3.0 METHODS

3.1 Introduction

The technique used in this study for the isolation of a recessive mutated lethal X chromosome has been used to demonstrate the relationship between X-irradiation dosage and mutation rate(1, 2). It involves the use of the so-called "Muller-5" marker chromosome which carries both dominant (Bar-eyed) and recessive (apricot-eyed) markers. This mutant X chromosome very infrequently crosses over with

the regular X chromosome. Therefore, if the regular (wild-type) X chromosome is affected by some agent in such a way as to be lethal in recessive condition, it will be transmitted without expression through the F_1 generation but will be expressed in the F_2 generation as an absence of wild-type males since the recessive (wild-type) F_2 males will not survive the pupal stage. All F_2 males will be Muller-5 in the event of a lethal mutation on the P_1 X chromosome whereas normally wild-type F_2 males could be expected to at least equal, if not exceed, on the average, Muller-5 F_2 males. The approximate rate of spontaneous lethal mutations is 0.0035 per X chromosome per generation. In 300 X chromosomes, one spontaneous mutation could thus be expected.

3.2 Materials

Two cultures of Drosophila melanogaster - a wild-type culture (Oregon-R) and a mutant culture (Muller-5) - were obtained from stocks maintained in the Department of Biology in The Catholic University of America.* In turn, these cultures were raised at Hazleton Laboratories to provide stocks for use in the study.

3.3 Apparatus

Details of the simulation apparatus used to provide the electromagnetic fields are contained in Chapter B of this report. In the present study, the flies to be exposed to EM fields were contained in 10 cm diameter x 15 cm long plastic tubes with nylon mesh ends. The tubes were oriented with their cylindrical axes parallel to the E-field lines, i.e., perpendicular to the E-field electrodes. Conventional sterile quarter-pint milk bottles were used to contain the flies before and after exposure. The flies were cultured in these bottles which contained about one inch of rearing medium composed of Cream of Wheat® (regular), unsulfured molasses, salt, water, and a mold inhibitor, Tegosept M®. The bottles were stoppered with sterile cotton. A four-power binocular microscope was used to sex and type flies.

3.4 Design

Three treatment groups of wild-type males were exposed in three replicates. All flies of a given replicate were exposed simultaneously. Flies were assigned to treatments randomly while lightly etherized.

* Courtesy of Prof. Sergei Polivanov

3.5 Treatments

Two EM frequencies were tested - 45 Hz and 75 Hz - each at a magnetic field strength of 2.0 gauss and at an electric field strength of 20 v/m in air. A control treatment, in which only the earth's magnetic field (approximately 0.6 gauss) and the ambient 60 Hz electric field (approximately 0.05 millivolts per meter) were present, was tested. The three treatments were made in three separate rooms. Breeding and culturing were performed in a fourth room.

3.6 Environmental Controls

Each of the three testing rooms and the breeding-culture room was maintained at $76^{o} \pm 2$ F during the study. Each room had separate thermostatic control and temperatures were monitored twice daily. Ambient room lighting in the treatment rooms was equated by photometric measurement. A nine hour on-15 hour off light cycle was maintained. Airflow through the rooms exchanged the volume 12 times per hour with 100% fresh air.

3.7 Procedure

3.7.1 - Two- to five-day old Oregon-R (wild-type) males were lightly etherized and transferred from stock bottles to plastic tubes for exposure. Each tube contained a watch glass filled with rearing medium. At the end of 48 hours exposure, 20 live males per EM treatment and 40 controls were selected for P_1 breeding in each replicate.

3.7.2 - Five exposed males were then placed in each breeding bottle which contained five virgin Muller-5 females. In each replicate there were four such group matings for each EM treatment and eight for each control treatment.

3.7.3 - Virgin Muller-5 females were obtained by clearing stock Muller-5 bottles of all emerged flies after etherizing and then collecting newly emerged flies two hours later. These were immediately sexed and only immature females were retained for use.

3.7.4 - As soon as numerous larvae appeared in the breeding bottles, the P_1 parents were removed and the larvae allowed to develop to adult fly stage. Two or three days later, after the F_1 flies had emerged, one F_1 heterozygous female and five F_1 Muller-5 males were placed together in each of 50 breeding bottles per EM treatment replicate and in each of 100 breeding bottles per control treatment replicate. As soon as F_2 larvae appeared in the bottle, the parents were removed and sex type confirmed.

3.7.5 - As the F_2 flies emerged in each bottle during an eight-day period, they were killed and categorized as to sex and male type (Muller-5 versus Oregon-R). The flies were then stored in vials containing 70% ethanol solution. Each F_2 breeding bottle (containing one F_1 female) was treated as one X chromosome for analysis and the fly counts were tabulated by bottle.

3.8 Observations

Flies were sexed by observation of the tip of the abdomen. Muller-5 males were distinguished from Oregon-R males in terms of eye-color and eye-shape. Homozygous Muller-5 females were distinguished from F_1 heterozygous females in terms of eye-color and eye-shape (see glossary). No attempt was made to observe visible and viable mutations on the X chromosome which would have appeared in the F_2 Oregon-R males; only lethal or "semi-lethal" mutations were sought. The latter were indicated by, in a successful F_2 culture, absence of or a minority of Oregon-R (wild-type) males, respectively, in a single F_2 culture. Sex ratios and ratios of wild-type to Muller-5 type males in the F_2 were determined for each successful individual culture. A successful culture was arbitrarily defined as one containing at least 12 live F_2 flies. Ratios of Muller-5 and heterozygous females were determined for each F_2 culture lacking wild-type males (and spot checked in 30 other F_2 cultures).

4.0 RESULTS

4.1 Mutation Frequencies

Table E1 indicates the number of successful F_2 cultures in which (a) no wild-type males were found and (b) less than half of the males were wild-type (Oregon-R). A total of 296 X chromosomes were analyzed for the control condition with 146 and 149 for the 45 Hz and 75 Hz conditions, respectively. Four, four, and one F_2 cultures, respectively, were not included in the analyses because of too small (<12) F_2 fly hatches.

No cultures lacking wild-type males were found among the control* F_2 cultures. Eight and seven were found in the 45 Hz and 75 Hz conditions, respectively. The probability of this difference in mutation frequency (with EM frequencies combined) occurring by chance is less than 1/1000 ($\chi^2 = 13.5$, df = 1). Twelve "semilethal" mutations were found among the

* One control culture contained no wild-type males and no females (and only three Muller-5 males). It was considered an unsuccessful culture.

control F_2 cultures. Five and two semilethals were found from the 45 Hz and 75 Hz conditions, respectively. These frequencies yielded $X^2 = 2.37$, df = 2, p >.30. Thus, no significance could be attributed to the differences in "semilethal" mutation frequencies.

4.2 Sex Ratios

Table E2 presents the mean numbers of each sex-type obtained in F_2 along with the percentage of males of each type. The most striking findings were (a) the mean sex ratios for cultures analyzed were each approximately the same between treatments with a very slight preponderance of females; (b) the mean sex ratios for the F_2 cultures lacking wild-type males were not greatly different between the 45 Hz and 75 Hz instances and, pooled, the ratio was the same as for the nonmutant cultures; and (c) there was a large highly significant reduction in the mean number of females in the 15 F_2 cultures lacking wild-type males which was due entirely to a lack of heterozygotes in 14 cultures and only one in the other. Table E3 shows the breakdown of female types in the 15 F_2 cultures lacking wild-type males, along with spot-checked 15 control and 15 exposed cultures. It can be seen that the yield of Muller-5 females in the "mutant" cultures was as high as in other "nonmutant" cultures.

5.0 DISCUSSION

The frequency with which F_2 cultures without wild-type males occurred following exposure of F_1 males to the EM fields was much greater than the normal mutation rate and significantly greater than following control treatment. On the basis of the test system's underlying genetic premises, it might be concluded that the results strongly indicate a mutagenic action of the EM fields. However, there was no *a priori* or physical reason to expect such an action from EM fields of such low strengths.

The mean number of F_2 females in the EM-exposed cultures lacking wild-type males was only about one-half that in the cultures with wild-type males (32.73 versus 61.35). On the other hand, the mean number of Muller-5 males in these respective cultures was approximately equal (32.20 versus 31.18). The sex ratios in the respective cultures were almost equal (50.9% versus 51.2% females). This unexpected result was due to a complete absence of heterozygous females which exactly corresponded to the lack of wild-type males in the "mutant" cultures, suggesting the action of a delayed dominant mutation or a procedural artifact.

The first replicate (100 exposed and 100 control F_2 cultures) and the third replicate contained all the cultures lacking wild-type males (10 and five, respectively). Thus, while not a consistent outcome, the result was not confined to one replicate.

In order for the result to be an artifact of a procedural mistake, it would have been necessary (a) to have used some nonvirgin Muller-5 females in the P_1 cross with "wild-type" males (so as to have had some Muller-5 females in F_1) and (b) to have crossed a few resulting F_1 Muller-5 females with their F_1 Muller-5 brothers to produce F_2 cultures with all Muller-5 offspring. While it is possible that one or the other of these mistakes could have occasionally occurred, the probability of both occurring with such frequency only in the exposed groups is very small. Muller-5 and heterozygous females are readily distinguished and the technique for insuring virginity in P_1 females was rigorous.

One F_2 culture from the 75 Hz treatment which contained no wild-type males but did contain one heterozygous female along with 16 Muller-5 females and 26 Muller-5 males, suggests either a spontaneous recessive lethal on the X chromosome or a partially dominant lethal induced by the agent.

While no instance is known of chemically induced mutations in Drosophila consequent on chemical alterations of culture media by EM radiation, the possibility of such an indirect mode of induction should not be overlooked.

While the sample sizes were insufficient to detect a weak mutagenic effect, e.g., a doubling of the spontaneous mutation rate, they were clearly large enough to detect a strong effect. However, due to the momentous biological implications of a strong dominant mutagenic effect from weak electromagnetic field exposure, acceptance of the present results as demonstrating such an effect would require replication in much larger samples. Also a dose-related effect should be demonstrated before concluding that ELF electromagnetic fields can be mutagenic.

6.0 APPENDICES

6.1 Tables of Data

Table E1 - Incidence of successful F_2 cultures (a) lacking wild-type (Oregon-R) males or (b) with wild-type comprising fewer than 50% of the males

	TREATMENT		
		45 HZ	75 HZ
	CONTROL	20 V/M, 2 GAUSS	20 V/M, 2 GAUSS
F_2 cultures analyzed	300	150	150
No. successful	296	146	149
No. without wild-type males	0	8	7
No. with <50% wild-type males	12	5	2

Table E2 - Mean number and percentage of each sex and type in F_2

	TREATMENT					
			45 HZ		75 HZ	
	CONTROL		20 V/M, 2 GAUSS		20 V/M, 2 GAUSS	
	M	%	M	%	M	%
All successful F_2 cultures:						
Females	50.2	51.1	54.7	51.9	65.0	50.5
Wild-type males	24.4	24.8	23.9	24.5	31.0	24.1
Muller-5 males	23.6	24.1	27.0	23.6	32.8	25.4
F_2 cultures without wild-type males:						
Females	-	-	23.8	53.8	49.1	48.0
Muller-5 males	-	-	20.4	46.2	53.3	52.0

Table E3 - Mean number and percentage of females of each type in selected F_2 cultures

	TREATMENT					
			45 HZ		75 HZ	
	CONTROL		20 V/M, 2 GAUSS		20 V/M, 2 GAUSS	
	M	%	M	%	M	%
Cultures without wild-type males:						
Muller-5	-	-	23.8	100	49.0	100
Heterozygotes	-	-	0	0	0.1	0
Spot checked cultures with wild-type males:						
Muller-5	27.3	49.3	39.5	61.0	32.1	46.6
Heterozygotes	28.1	50.7	25.3	39.0	37.4	53.4

6.2 Glossary

1. Muller-5 males (all homozygous): Bar- and white-apricot-eyed.
2. Homozygous Muller-5 females: Bar- and white-apricot-eyed.
3. Heterozygous Muller-5 females: Modified Bar-eyed but with normal red eyes.
4. Modified Bar: Eye slightly smaller than normal and shaped like a kidney bean.
5. Oregon-R wild type: Normal eyes - oval and red.
6. Recessive lethal mutation: Expressed by absence of wild-type males in F_2.
7. Dominant lethal mutation: Expressed by presence only of Muller-5 flies in F_2.

6.3 References

1. Muller, H. J., Herskowitz, I. H., Abrahamson, S., and Oster, I. I., Genetics, 39, 741-749, 1954.

2. Spencer, W. P., and Stern, C., Genetics, 33, 43-74, 1948.

CHAPTER F
BACTERIA MUTAGENESIS STUDY

Richard A. Pledger
William B. Coate

TABLE OF CONTENTS

1.0 INTRODUCTION

Chapter F contains the results of a bacteria mutagenesis study performed in fulfilling the requirements of the Naval Electronic Systems Command, Contract No. N000-39-69-C-1572. The objective of this contract was to assess preliminarily the possibilities of biological/ecological impact from the continuous presence in a given locale of electric fields, magnetic fields, and earth return currents of specified magnitudes. The present pilot study was performed to assess the ability of these electromagnetic fields to induce auxotrophic mutants in a bacterial population.

2.0 SUMMARY

Escherichia coli, strain B, was selected as a representative bacterium because of the historical data generated with this microbe with respect to induction of auxotrophic mutants. Cultures of pure prototrophs were subjected to the environmental fields for 24 hours after which the relative number of mutants were determined. These data were compared to control cultures and populations subjected to ultraviolet radiation. Whereas a significant number of auxotrophs could be determined after UV irradiation, no mutagenic effects were observed in those cultures subjected to the electromagnetic fields used in this study.

3.0 METHODS

3.1 Introduction

The basis of this experimental design is that a prototrophic or wild-type *Escherichia coli* cell is able to grow (i.e., multiply) in a minimal medium composed of glucose and inorganic salts. All carbon and nitrogen containing constituents of the cell, e.g., vitamins, amino acids, growth factors, etc., can be synthesized from glucose as a carbon and energy source and the ammonium ion as a nitrogen source. An auxotrophic mutant, i.e., a cell requiring a specific additional nutrient, will not be able to grow in this medium. A mixture of proto- and auxotrophs can be separated by utilizing a characteristic of penicillin, namely, that this antibiotic will kill only actively growing cells. In a medium in

which auxotrophs are unable to grow, the mutants will not be substantially affected(1).

Populations subjected to potential mutagens may be compared with control populations for frequency of auxotrophs.

3.2 Materials

3.2.1 Test Organism

E. coli, strain B, was obtained from the American Type Culture Collection (ATCC 11303). This wild-type, penicillin sensitive strain is capable of multiplying in a minimal growth medium.

3.2.2 Media

3.2.2.1 Liquid Minimal Medium (LMM)

K_2HPO_4	7.0 g	$Mg\ SO_4 \cdot 7H_2O$	0.1 g
KH_2PO_4	3.0 g	Na citrate$\cdot 3H_2O$	0.5 g
$(NH_4)_2\ SO_4$	1.0 g	glucose	2.0 g

Distilled H_2O 1000 ml

pH 7.0 after sterilization

3.2.2.2 Solid Minimal Medium (SMM)

LMM + 1.5% Agar

3.2.2.3 Penicillin Treatment Medium (PTM)

LMM + 0.6 M sucrose and 0.01 M $Mg\ SO_4 \cdot 7H_2O$

3.2.2.4 Complete Growth Media

Liquid: Tryptic SO_4 Broth (TSB) Difco

Solid: Tryptic SO_4 Agar (TSA) Difco

3.2.2.5 Restrictive Medium (RM)

One part TSA and 19 parts SMM

3.3 Apparatus

Plexiglas® culture vessels were specially constructed for this study. Each vessel was 3 cm high, 3 cm wide, and 14.5 cm long (inside dimensions). The ends were fitted with a Nu-way® stud which was filed flush with the inside surface which was then coated with a mixture of graphite and styrofoam dissolved in ethylene dichloride. This coating provided low (<400 ohms) resistance electrodes when the studs were wired to a voltage source. Each vessel had a lid with two small holes through which the liquid medium could be inoculated. These were plugged with sterile cotton after inoculation. Prior to use the culture vessels were sterilized for 12 hours in an Anprolene Sterilizer Model 2270 with 84% ethylene oxide.

Details of the apparatus used to provide the magnetic fields are contained in Chapter B of this report.

3.4 Design

Twelve identical culture vessels were inoculated with *E. coli* B cells in IMM. They were then randomly assigned to six groups, two replicate vessels per group, as follows:

a) 45 Hz, 10 v/m, 1 gauss
b) 45 Hz, 20 v/m, 2 gauss
c) 45 Hz, Control
d) 75 Hz, 10 v/m, 1 gauss
e) 75 Hz, 20 v/m, 2 gauss
f) 75 Hz, Control

3.5 Treatments

3.5.1 - Two EM frequencies were tested - 45 Hz and 75 Hz - each at two electric field strengths - 10 volts per meter and 20 volts per meter - and at two respective magnetic field strengths - 1.0 gauss and 2.0 gauss - concurrently. Because the bacteria were suspended throughout the IMM in each vessel, they were exposed to approximately 5.0 milliamperes of electric current passing through the high conductivity medium.

The control groups were exposed only to the earth's magnetic field (approximately 0.6 gauss) and the ambient 60 Hz electric field (approximately 0.05 millivolts per meter) in the two respective ELF test rooms at the same time as the other groups.

The inoculated vessels were exposed to their respective treatments for 24 hours.

3.5.2 - The *E. coli* B culture used to inoculate the above vessels was also used to demonstrate the successful application of the technique for isolation of mutants. Three 14 1/2 cm diameter glass petri dishes were each inoculated with 10 ml of a cell suspension having 2.7×10^9 cells/ml. One of these cultures was irradiated for 60 seconds at a distance of 45 cm with ultraviolet radiation from a F8T5D General Electric tube reported to yield predominantly 2540 Å rays with an intensity of approximately 17 microwatts per square cm at one meter. This, however, was not experimentally confirmed. A second vessel was similarly irradiated but for 90 seconds. The unirradiated third culture served as a control.

After assay to quantify surviving cells, 5 ml of each culture was inoculated into 100 ml of TSB and the mixture was incubated at 37° C for 18 hours to generate high titered populations. From this point on, these cultures were processed as described in Section 3.7.2.

3.6 Environmental Controls

3.6.1 - The rooms containing the simulators in which exposures of the EM fields were made were maintained at 76° ± 3 F during the exposure. Each room had separate thermostatic control and the temperatures were continuously recorded throughout the exposure period. The 45 Hz room fluctuated between 76° and 79° during this period while the 75 Hz room had but a one-degree swing.

Although the ambient room illumination was equated by photometric measurement, this factor was eliminated by the fact that the culture vessels were wrapped in opaque plastic during their residence in the simulator rooms.

3.6.2 - The postexposure bench-top procedures were carried out under subdued illumination (room lights off, red lamp on) in the bacteriology laboratory. All procedures were carried out on all treatment conditions concurrently in the same room, except that the positive control study was somewhat out of phase with the main study. Temperature controls were provided by incubators as required; otherwise room temperature prevailed.

3.7 Procedure

3.7.1 - Immediately prior to placement in the test rooms for treatment, each Plexiglas® culture vessel was filled with 50 ml of LMM and then inoculated with a suspension of purified *E. coli* B cells to yield approximately 22 cells per ml after rocking to

distribute the cells. Following the 24-hour exposure to their respective treatments the contents of each vessel were concentrated by centrifugation, resuspended in 5 ml of TSB, and incubated at 37° C for three hours.

3.7.2 - Five ml of the cell culture from each vessel (including those in the UV positive control test) was concentrated by centrifugation, washed twice with IMM to remove extracellular metabolites and resuspended in IMM. One ml of each washed suspension was inoculated into 9 ml of PTM and the resulting suspension was incubated at 37° C for three hours in order to permit cell utilization of intracellular metabolites which may permit growth and multiplication in subsequent steps.

After incubation, 1000 I.U. of penicillin was added to each culture, and incubation was continued for three additional hours during which the wild-type cells were subjected to the cidal action of the antibiotic. The culture was subsequently centrifuged and resuspended in 5 ml of a 100 unit/ml aqueous solution of penicillinase. Dilutions of the resulting suspensions were then plated onto RM, a medium designed to possess limited growth promoting factors required by the mutants.

3.8 Observations

After incubation, presumptive mutants within the residual wild-type population were identified by visual observation of small colonies failing to develop fully as a result of limited nutrients provided by the minimal quantity of TSA incorporated into the RM medium.

A total of 264 small colonies from each test condition was examined to confirm the dependence upon externally supplied growth factors. Suspected mutant colonies were streaked onto both SMM and TSA. After incubation at 37° C for 24 hours, those colonies which grew only on TSA were reexamined for such dependence. Mutants were not considered confirmed until such reexamination demonstrated their failure to grow on SMM.

4.0 RESULTS

4.1 Mutation Frequencies

The table presents the frequencies of auxotrophic mutations identified and confirmed in each of the various samples derived from the populations exposed to each condition of the experiment. These frequencies were submitted to a series of chi-square analyses with results as follows:

4.1.1 - The data from Tests 1-12 yielded a χ^2 of 15.32, which with 11 degrees of freedom (df) is not significant ($p = .17$). Thus, no mutagenic effect of EM fields was demonstrated. Actually, the control tests had the preponderance of the few mutations observed.

4.1.2 - The data from Tests 13-15 (positive control study) gave a $\chi^2 = 4.72$, df = 2, $p = .09$ which was not quite significant. Thus, by itself this study did not succeed in demonstrating the mutagenic effect of ultraviolet radiation.

4.1.3 - The data from the 60-second UV exposure obtained a week earlier to validate the technique used in the main study, when compared to control yielded a $\chi^2 = 24.27$, df = 1, $p < .001$. Thus, it can be fairly said that the *E. coli* B strain from which the exposed cultures were taken was mutagenically susceptible.

4.1.4 - When all the data from Tests 1-12 plus 15 were analyzed against Tests 13 plus 14, a $\chi^2 = 20.85$, df = 2, $p < .001$ was obtained, indicating that when compared against the large population (Tests 1-12 and 15) in which no mutagenic effect was shown, Tests 13 and 14 did yield a significant increase in mutations.

4.2 Growth Rates

4.2.1 - The 24-hour titres of the cultures exposed to the various treatments are also shown in the table. While a trend existed for the cultures exposed to the EM fields to show higher titres than the controls, this trend was not consistent nor significant by the Mann-Whitney U-test ($U = 10$, $n_1 = 8$, $n_2 = 4$, $p = .18$). Thus, no cidal nor growth inhibiting effect was observed; a possible growth-promoting effect proved insignificant.

4.2.2 - The exposures to ultraviolet light proved to be highly cidal in a dose-related manner.

5.0 DISCUSSION

The results of this experiment indicated that, within the limits of sensitivity of the method, ELF electromagnetic fields as high as 20 v/m, 2.0 gauss did not have a mutagenic effect on *E. coli* B bacteria exposed for 24 hours in a conductive medium. That the method (i.e., the B strain) was sensitive to a known mutagenic radiation was shown by the frequency of auxotrophic colonies following a 60-second exposure to ultraviolet light. Failure to increase auxotrophs following 90-second UV exposure was probably due to the highly cidal effect of this treatment.

No consistent effect on growth rate was observed as a function of exposure to ELF fields. In some exposed cultures, titres were higher and in others, lower than in corresponding control cultures. Thus, these fields were neither highly cidal, inhibiting, nor growth promoting. However, overall, the exposed cultures average a higher titre than the controls. Two preliminary studies using slightly higher concentrations of inocula showed the same trend. While the trend in the main study was statistically insignificant, the fact that it was replicated three times probably indicates a reliable effect. This could probably best be accounted for as due to a thermal artifact of the current passing through the slightly resistive medium. The potential ecological significance of such a finding is questionable. Growth promotion by a thermal factor in a confined space does not lead directly to a prediction of bacterial overabundance in unconfined space.

6.0 APPENDICES

6.1 Table of Data

Summary of Results of Tests for Mutagenicity

TEST NO.	ENVIRONMENTAL CONDITION	REPLI-CATE	TITER AFTER EXPOSURE cells/ml	NO. COLONIES EXAMINED	NO. CONFIRMED MUTANTS
1	45 Hz - 10 v/m	1	4.0×10^6	264	0
2	1 gauss	2	8.7×10^6	264	0
3	45 Hz - 20 v/m	1	4.4×10^6	264	1
4	2 gauss	2	2.7×10^6	264	0
5	Control for	1	7.6×10^5	264	0
6	45 Hz treatments	2	1.4×10^6	264	2
7	75 Hz - 10 v/m	1	4.1×10^5	264	0
8	1 gauss	2	4.2×10^5	264	0
9	75 Hz - 20 v/m	1	8.0×10^5	264	0
10	2 gauss	2	7.7×10^5	264	0
11	Control for	1	6.4×10^5	264	2
12	75 Hz treatments	2	6.6×10^5	264	2
13	60 sec. UV exposure	-	1.1×10^4	264	6
14	90 sec. UV exposure	-	4.5×10^2	264	2
15	Control for UV exposure	-	2.7×10^9	264	1
16*	60 sec. UV exposure	-	-	25	4

* Data for Test 16 derived during the week preceding Tests 1-15, but utilizing the described technique without alteration

6.2 Glossary

Prototroph - Cell that possesses the ability to grow (i.e., multiply) in media containing a minimum of growth factors

Auxotroph - Cell which requires growth factors in addition to those necessary for prototrophs to sustain growth

Cidal - Possessing the ability to kill, as opposed to inhibit, growth

Wild-type - Prototroph

Mutant - Auxotroph, herein

Mutation - A well-marked variation from parent to offspring due to chromosomal or gene alteration

Colony - A population of bacterial cells derived from a single cell restricted in growth to a delimited area on a solid growth medium

6.3 Reference

Lichstein, Herman C., and Oginsky, Evelyn L., Experimental Microbial Physiology, San Francisco: W. H. Freeman and Co., pp. 73-76, 1965.

CHAPTER G
PLANT CYTOGENETIC STUDY

William B. Coate
Smith Sae Hoo

TABLE OF CONTENTS

1.0 INTRODUCTION

Chapter G contains the methodology and results of a cytogenetic study, using onion root tips, performed in fulfilling the requirements of the Naval Electronic Systems Command, Contract No. N000-39-69-C-1572. The objective of this contract was to assess preliminarily the possibilities of biological/ecological impact from the continuous presence in a given locale of electric fields, magnetic fields, and earth return currents of specified magnitudes. The present study was performed as a pilot study to determine whether chromosomal damage could be expected in plants exposed to these levels of electromagnetic (EM) fields and/or earth return currents at extremely low frequencies (ELF).

2.0 SUMMARY

Onion sets were allowed to root out in water in which electrodes produced a 10- or 20-volt per meter electric (E-) field. The glass or wooden vessels containing the water were situated in 1.0 and 2.0 gauss magnetic fields, respectively. Both 45 Hz and 75 Hz EM fields were tested along with a control condition. The root tips were harvested after 72 hours' exposure, fixed and stained, made into squash preparations, and microscopically examined for the presence of mitotic figures and for polyploidy, anaphase or telephase lags or bridges, and other structural aberrations in the figures. One to two hundred mitotic figures from each treatment were examined. No effects were observed attributable to EM fields.

3.0 METHODS

3.1 Introduction

The onion root-tip preparation for the assessment of chromosomal damage due to environmental agents is classic(1). Water plants and terrestrial plants may, of all organisms, have the greatest consistent exposure to SANGUINE EM fields. It was thought that choice of this model would provide a sensitive indicator of potential cytogenetic damage.

3.2 Materials

Small onions (sets), Allium cepa, were obtained from a local garden supply house in batches as needed.

3.3 Apparatus

3.3.1 - Details of the simulation apparatus used to provide the electromagnetic fields are contained in Chapter B of this report. In the present study, two types of water vessels, over which to root out the onion bulbs, were used: a polyvinyl plastic sheet-lined pine box and a glass battery jar. In both cases a pair of stainless steel electrode arrays each consisting of horizontal rods welded to a vertical rod were submerged to the level of the top rod with an ELF voltage impressed appropriate to the distance between electrodes. The bulbs were set in holes in sheet Plexiglas® set above the water so that their lower portions were in contact with the tap water. The vessels with the bulbs were placed in appropriate magnetic fields in space-time quadrature with the electric fields in the water.

3.3.2 - A Leitz Wetzlar light microscope was used to examine slide preparations.

3.4 Design

Due to technical difficulties (see 4.2) during the conduct of this study, no straightforward design was in fact used. Onion bulbs were rooted out under each of the five treatment conditions at various times, sometimes concurrently, sometimes not. However, control bulbs were always rooted concurrently with any EM treatment bulbs.

3.5 Treatments

Two EM frequencies were tested - 45 Hz and 75 Hz - each at two electric field strengths - 10 and 20 volts per meter - and at two respective magnetic field strengths - 1.0 and 2.0 gauss - concurrently. A control treatment, in which only the earth's magnetic field (approximately 0.6 gauss) and the ambient 60 Hz electric field were present, was also tested. The 45 Hz treatments were applied in one room, the 75 Hz in another, and the control in a third.

3.6 Environmental Controls

Each of the three treatment rooms was maintained at $76^{\circ} \pm 2$ F air temperature during the study. Water

temperature was monitored twice a day during treatments and varied between 73° and 74° F. Ambient room lighting was equated by photometric measurement. A nine hour on-15 hour off light-dark cycle was maintained. Airflow through the rooms exchanged the volume 12 times per hour with 100% fresh air. Tap water was aged for at least 24 hours prior to use.

3.7 Procedure

3.7.1 - Either 16 or eight onion sets were placed on perforated Plexiglas® sheets over water in a common vessel and subjected to a given treatment simultaneously. At the end of a 72-hour exposure the roots were harvested without allowing them to become dry and all those from a given treatment placed in Carnoy's fixative overnight. After washing in 90% ethanol they were stored in 90% ethanol until stained in Feulgen's reagent. After three washes in SO_2 water baths, three randomly selected root tips from a single treatment were placed together on a glass slide and subjected to the routine for squash preparations. Nine slides were prepared for each treatment.

3.7.2 - Suitably coded slides were examined in a single-blind procedure for suitable mitotic spreads under the high dry objective 80x of the microscope. Up to 50 spreads per tip which were judged to have good chromosomal morphology were analyzed in detail under oil immersion for gross chromosomal changes.

3.8 Observations

3.8.1 - Root growth was monitored daily and only those bulbs showing healthy "normal" root growth were used in this study. The length of the longest root from each bulb was measured to the nearest millimeter prior to harvesting. Curvature and coloration were noted.

3.8.2 - The cells in the slide preparations were examined for polyploidy, anaphase and telephase lags, anaphase and telephase bridges, and any other structural aberrations that might appear.

3.8.3 - A representative sampling of roots and root tips which appeared to have developed abnormally were suitably prepared for sectioning and examined microscopically for evidences of morphological aberrations and for frequency of mitotic figures.

4.0 RESULTS

4.1 - Table G1 presents the main results of this study. No effects attributable to EM field exposures were noted.

4.2 - During preliminary studies a growth retardation effect was observed in all roots of onion sets exposed in water with 10 or 20 v/m at 45 Hz in the water in a 1.0 or 2.0 gauss magnetic field and also of bulbs exposed to 2.0 gauss alone at 75 Hz. In the higher field strengths the roots were stunted and curved abnormally with brown tips. Additional sets of eight bulbs were rooted in water which had been removed from the plastic-lined wooden exposure boxes and placed in vitreous enamel pans. These also showed a similar but smaller root-growth retardation as compared to controls. New water was placed in the 45 Hz, 20 v/m, 2.0 gauss exposure box (and in the control box) and the effect was replicated. Glass battery jars were equipped with electrodes in aged tap water and new onion sets rooted out in order to determine the source of this effect. (Meanwhile, microscopic examination of representative Feulgen-stained root tips from stunted, retarded, and normal roots revealed a total lack of mitotic figures in the first two and an abundance in the last category.) Five separate replications were made using either 10 v/m, 1.0 gauss or 20 v/m, 2.0 at 45 Hz or 10 v/m, 1.0 gauss at 75 Hz, with concurrent control groups. Eight bulbs were rooted out in each glass container. Since the results of these replications were in opposition to the previous results, *i.e.*, the controls were uniformly retarded relative to the EM treatments in four out of the five replications, the problem of root-growth effects resolved itself into that of why all roots of bulbs rooted out together in one vessel of water should be stunted or none be stunted regardless of the FM environment. Since this question lay outside the scope of the project, no further attention was given to it.

5.0 DISCUSSION

The chromosomal analysis of grossly normal-appearing root tips of onion bulbs exposed to various combinations of electric and magnetic fields at 45 and 75 Hz gave no indication of aberrations due to these EM fields. It was elected to examine only normal-appearing root tips after the growth retardation and other gross effects described in Section 4.2 had been determined to be artifactual with respect to EM fields; artifacts whose explanation(s), however, were not determined. (The most likely hypothesis was that one or more of the bulbs in a group, rooted out together, chemically contaminated the water in which they were grown, resulting in the observed uniform effects on all the roots in that water. The nature of the "contaminant" remained unknown.)

6.0 APPENDICES

6.1 Table of Data

Table G1 - Chromosome aberrations in Allium cepa root tips

TREATMENT	OBSERVATIONS			
	NO. ROOT TIPS	NO. CELLS EXAMINED	NO. ABERRATIONS*	% ABERRATIONS
Control	24	218	0	0
45 Hz, 10 v/m, 1 gauss	24	229	2	0.87
45 Hz, 20 v/m, 2 gauss	18	133	2	1.5
75 Hz, 10 v/m, 1 gauss	21	221	0	0
75 Hz, 20 v/m, 2 gauss	27	293	0	0

* Anaphase or telephase lags or bridges

6.2 Reference

Sax, K., Behavior of x-ray-induced chromosomal aberrations in onion root tips, Genetics, 26, 418, 1941.

CHAPTER H
SEED GERMINATION AND EARLY GROWTH STUDY

William B. Coate

TABLE OF CONTENTS

1.0 INTRODUCTION

Chapter H contains the methodology and results of a seed germination and early growth study performed in fulfilling the requirements of the Naval Electronic Systems Command, Contract No. N000-39-69-C-1572. The objective of this contract was to assess preliminarily the possibilities of biological/ecological impact from the continuous presence in a given locale of electric fields, magnetic fields, and earth return currents of specified magnitudes. The present study was performed as a pilot study to determine whether gross effects on seed germination or early growth could be expected from these levels of electromagnetic fields and/or earth return currents at extremely low frequency (ELF).

2.0 SUMMARY

Buckwheat seeds and sunflower seeds, both dicotyledons selected as rapid germinators with water penetrable covers, and corn seeds similarly chosen as a monocotyledon, were row planted in premoistened milled sphagnum moss with resistivity approximately 2000 meter-ohms and exposed to ELF electromagnetic fields of selected strengths. The cumulative number of seeds visibly germinating were recorded daily for nine days. The length of shoot of each seedling was measured seven days after the first germination of its species. During the entire study period, temperature, humidity, and light cycles and intensities were maintained constant and equalized over the electromagnetic conditions.

3.0 METHODS

3.1 Introduction

Mericle, et al.,(1) have shown that shoot length is a sensitive indicator of constant magnetic field effect on barley seedling growth. Exposure to a maximum field intensity of 1200 oersteds for 93-185 hours significantly increased shoot length without having affected germination rate.

Inasmuch as the seeds in the present study were to be exposed to both a low sinusoidal magnetic field and a low sinusoidal electric field in a conductive medium, measurement of both germination rate and shoot growth were deemed desirable. No previous studies of possible ELF effects on these parameters have been noted.

3.2 Materials

A total of 450 sunflower seeds obtained from a biological supply house (Wards) were randomly divided into five groups corresponding to five electromagnetic field conditions. Similarly, 450 corn (Golden Cross Bantam) seeds and 770 buckwheat seeds obtained from a local supply house (Southern States Farm Cooperative) and Wards, respectively, were each randomly divided into the five treatment groups.

3.3 Apparatus

Details of the simulation apparatus used to provide the electromagnetic fields and to contain the biological materials tested are contained in Chapter B of this report. In the present study, the seeds were planted in premoistened milled sphagnum moss (Nodampoff®) obtained from a supply house. The moss was squeezed as dry as possible after soaking and placed to a depth of 10 centimeters in six adjacent cells of five 50 x 122 cm pine boxes containing seven vertical stainless steel electrode arrays which provided the horizontal electric field. The electrodes were in contact with the moss as well as protruding above the surface. Potential differences were impressed on alternate electrodes to provide the appropriate voltage gradients in the cells. The alternating magnetic fields were developed from a set of current carrying coils surrounding each pine box. The resulting electric and magnetic fields were generated in space-time quadrature. The resistivity of the moss in the boxes was 2000 ± 100 meter-ohms during the study. The control box was fitted with identical electrodes but no voltage was used.

3.4 Design

Each of the five treatment groups of each type of seed was subdivided into two groups to be planted on separate days. These were treated as replicates of the basic design. All seeds were planted between 10:00 and 11:30 a.m.; all seeds of a given replicate of a given species were planted on the same day in a treatment order determined by chance.

3.5 Treatments

Two EM frequencies were tested - 45 Hz and 75 Hz - each at two electric field strengths - 10 volts per meter and 20 volts per meter - and at two respective magnetic field strengths - 1.0 gauss and 2.0 gauss - concurrently. A control treatment, in which only the earth's magnetic field (approximately 0.6 gauss) and the ambient 60 Hz electric field (approximately 0.05 millivolts per meter) were present, was

tested. The 45 Hz treatments were tested in one room, the 75 Hz treatments in another, and the control in a third.

3.6 Environmental Controls

Each of the three testing rooms was maintained at 76° ± 2 F during the study. Each room had separate thermostatic control and temperatures were monitored twice daily. Ambient room lighting was equated by photometric measurement. Each pine box was specially illuminated by the light from two 40-watt Gro-Lux® "sunlight" fluorescent lamps suspended 42 inches above. A 12-hour light cycle was maintained. Airflow through the rooms exchanged the volume 12 times per hour with 100% fresh air.

3.7 Procedure

Seeds were planted in rows in 20 cm x 30 cm plots (see Figure H1) inside pine boxes. Buckwheat seeds were planted at 2.5 cm intervals while sunflower and corn seeds were planted at 3.0 cm intervals. All seeds were planted a depth approximately twice their minimum diameter and lightly tamped in by hand. As soon as planting was completed within a plot, the appropriate electrical field was applied (except for controls). The magnetic field was present during planting. The boxes were covered with a sheet of transparent polyvinyl immediately after seed planting. The covering was removed just prior to the measurement of shoot lengths (see below).

3.8 Observations

3.8.1 - Observations were made daily, at the same hour that plantings had been made, to determine and record the total number of visibly germinated seeds in each plot. A visible germination was counted whenever any portion of the emerged seedling was visible without moving moss.*

3.8.2 - All germinated seeds were observed to determine whether they developed to the point of leaves separating from the stem shoot.

---

* In some instances it seemed obvious that the seed had germinated, even though no seedling was visible. In such cases it was arbitrarily decided not to count the germination in order to avoid the bias of expectation in cases where moss lumps might be due to root sprouts from other seeds.

3.8.3 - The lengths of shoots of all seedlings which reached the leafing-out stage were measured *in situ* to the nearest 0.5 centimeter seven days after germination, if alive. These data were analyzed by Student's t-test for significance of differences between independent means.

3.8.4 - Mortality of seedlings was recorded.

4.0 RESULTS

4.1

Table H1 presents the percentage of seeds which germinated on each of six days following planting. The results are combined for the two replicates since no appreciable differences were observed between them. No new germinations were observed after six days in any species. No overall ELF effects on germination rate or germination success were obtained although on Day 2 and Day 3 there was uneven germination across conditions.

4.2

All seeds but one corn seed which germinated lived to develop leaves separate from the stem.

4.3

Table H2 presents the mortality percentages at seven days postgermination of the seedlings which reached the leafing-out stage of development. No consistent effects were noted.

4.4

Table H3 presents the mean shoot lengths in centimeters of the seedlings seven days after the start of germination. Included are the standard deviations of the distributions from which the means were computed. There were no ELF related effects on growth rate of buckwheat or corn seedlings, although the mean of the 45 Hz 10 volts per meter, 1.0 gauss corn seedlings was significantly smaller than the control mean. All eight of the plots of sunflower seedlings exposed to ELF electromagnetic fields showed significant ($p < .001$) growth retardation as compared to the combined control plots.

5.0 DISCUSSION

5.1

The results of this study are not easily interpreted. While it was clear that no ELF effects were obtained on germination or viability of seedlings in the three species tested, there was evidence suggesting possible retardation of growth in the sunflower seedlings caused by exposure to as low strength fields as 10 volts per meter in a conductive medium and/or 1.0 gauss at 45 Hz and 75 Hz. However, the lack of a "dose-related" effect, i.e., no greater retardation from exposure to a 20 volts per meter electric field plus a 2.0 gauss magnetic field, raises the question of a possible artifact causing the obtained differences. Of course, it is possible that the differences in field strengths were insufficient to produce detectable growth differences, even though the lower strength field or fields was the cause of the retarded growth. The possibility of an artifact cannot be ruled out, but the fact that no similar differences were obtained with the other two species, grown in adjacent plots under the same environmental conditions, renders an artifactual explanation doubtful. Ideally, each treatment condition, including control, would have been randomly applied to plots within a given culture box. However, this was not physically possible with the magnetic field simulator employed. A practical compromise had to be made which undoubtedly renders the results suspect. Whether it is possible to conduct a valid study in which both electric and magnetic fields are simultaneously applied is questionable. Clearly, additional experimental work should be done to verify the ostensible growth effect on sunflower seedlings (and the lack of effect on the other two species). The present study suggests that an effect may have occurred but a more sophisticated design and larger sample sizes are needed to resolve the empirical doubts about its reality. In any case, it should be recognized that the electric and magnetic field strengths employed in this study were at least 150 and five times greater, respectively, than those expected at the earth's surface from a SANGUINE antenna. It would be incorrect to infer from the present data that such an antenna would have any effects on plant growth.

6.0 APPENDICES

6.1 Tables of Data

Table H1 - Cumulative percentage of seeds germinating on each of five days postplanting

			TREATMENT			
			45 HZ		75 HZ	
SPECIES	DAY	CONTROL	10 V/M 1.0 GAUSS	20 V/M 2.0 GAUSS	10 V/M 1.0 GAUSS	20 V/M 2.0 GAUSS
Buckwheat	1	0	0	0	0	0
	2	16.2	6.5	0	0.7	6.5
	3	73.4	62.4	60.4	70.8	41.6
	4	78.5	76.7	77.9	83.1	87.7
	5	80.5	79.2	80.5	87.0	88.3
	6	80.5	79.2	80.5	87.0	88.9
Sunflower	1	0	0	0	0	0
	2	0	1.1	2.2	0	0
	3	22.2	15.6	20.0	3.3	25.6
	4	60.0	55.6	67.8	44.4	78.9
	5	60.0	60.0	77.7	65.6	87.8
	6	64.4	60.0	77.7	74.7	87.8
Corn	1	0	0	0	0	0
	2	0	2.2	1.1	0	0
	3	62.0	78.9	67.8	41.1	17.8
	4	96.7	92.2	93.3	91.1	94.4
	5	97.8	95.5	94.4	95.5	97.8
	6	98.9	95.5	95.5	97.8	98.9

Table H2 - Mortality percentages at seven and 14 days postgermination of seedlings reaching the leaf stage

	TREATMENT				
		45 HZ		75 HZ	
SPECIES	CONTROL	10 V/M 1.0 GAUSS	20 V/M 2.0 GAUSS	10 V/M 1.0 GAUSS	20 V/M 2.0 GAUSS
Buckwheat	23.9	24.3	12.7	1.5	4.4
Sunflower	3.4	0	8.2	0	3.5
Corn-7 days	0	1.2	0	0	1.1
-14 days	13.9	3.7	2.4	20.3	7.9

Table H3 - Mean (M) and standard deviation (SD) of the lengths (in centimeters) of seedling shoots measured seven days postgermination

	TREATMENT				
		45 HZ		75 HZ	
SPECIES	CONTROL	10 V/M 1.0 GAUSS	20 V/M 2.0 GAUSS	10 V/M 1.0 GAUSS	20 V/M 2.0 GAUSS
Buckwheat	M = 12.1	M = 13.0	M = 12.4	M = 12.0	M = 13.0
	SD = 1.3	SD = 1.1	SD = 2.1	SD = 2.0	SD = 2.0
Sunflower	M = 10.4	M = 8.9*	M = 8.4*	M = 7.1*	M = 8.2*
	SD = 1.1	SD = 0.9	SD = 0.9	SD = 0.9	SD = 0.9
Corn	M = 11.0	M = 9.7*	M = 11.1	M = 11.0	M = 12.4
	SD = 0.7	SD = 0.5	SD = 0.7	SD = 1.6	SD = 1.4

* Less than control, $p < .001$

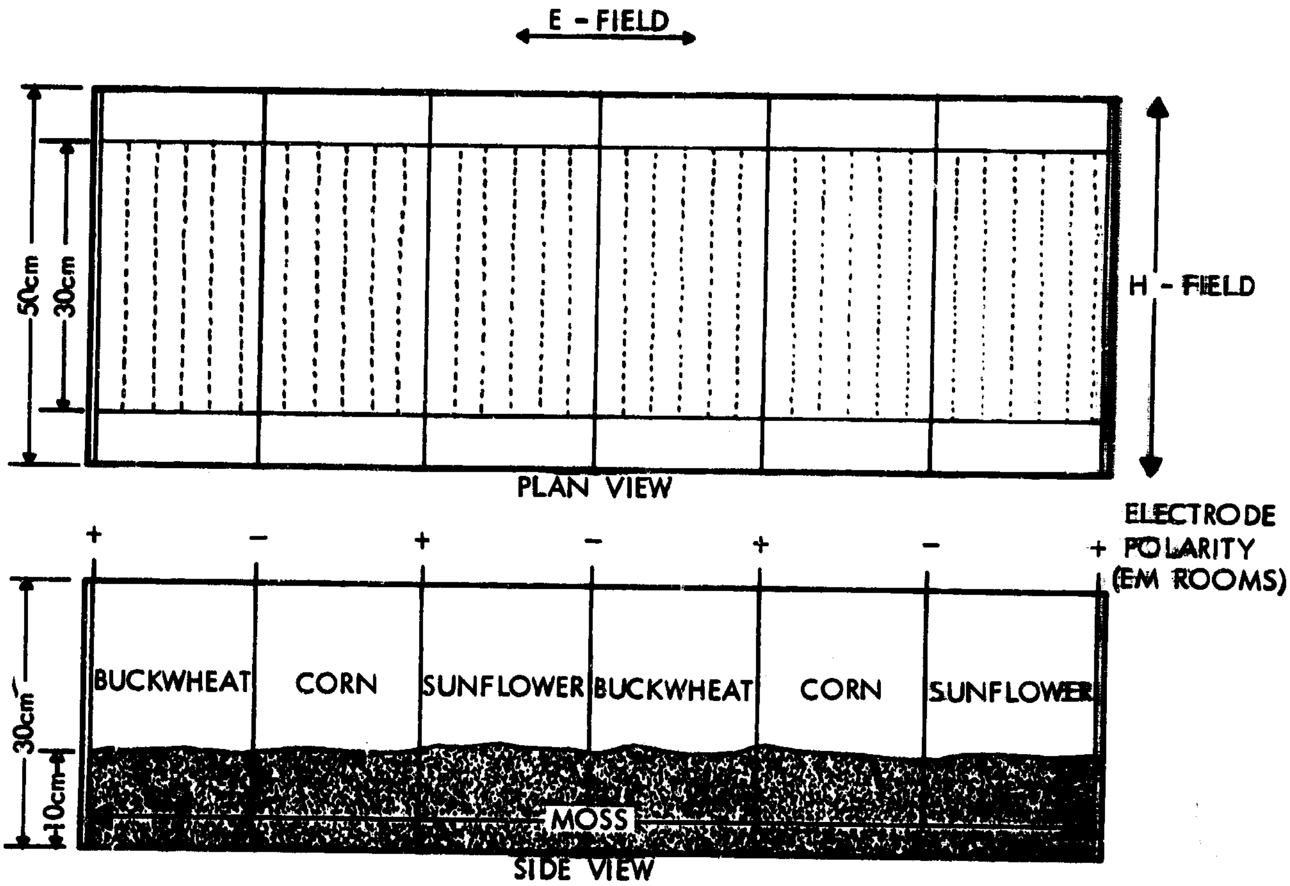


Figure H1 - Layout of seed plots in each pine box used in germination study

6.2 Reference

Mericle, R. P., Mericle, L. W., Smith, A. E., Campbell, W. F., and Montgomery, D. J., in Biological Effects of Magnetic Fields, M. F. Barnothy, Ed., p. 183-195, Plenum Press, New York, 1964.

6.3 Glossary

Dicotyledon - Subclass of angiospermous plants, having two seed leaves. Most deciduous trees, herbs, and shrubs are dicotyledonous.

Monocotyledon - Subclass of angiospermous plants, having a single seed leaf. Grasses (including wheat and corn) and lilies are monocotyledonous.

Angiospermous - Having seeds in a closed ovary.

CHAPTER I

ELECTRICAL FIELD PERCEPTION AND PREFERENCE STUDIES

William B. Coate

TABLE OF CONTENTS

1.0 INTRODUCTION

Chapter I contains the methodology and results of multiple species preference tests performed in fulfilling the requirements of the Naval Electronics Systems Command, Contract No. N000-39-69-C-1572. The objective of this contract was to assess preliminarily the possibilities of biological/ecological impact from the continuous presence in a given locale of electric fields, magnetic fields, and earth return currents of specified magnitudes. The present study was performed as a pilot study to determine whether various animals could "perceive" and/or would tend to leave, stay in, or behave indifferently to, electric fields and/or earth return currents at extremely low frequencies (ELF).

2.0 SUMMARY

Fingerling bluegill fish, mallard ducklings, and Eastern painted turtles were preference-tested in water; turtles, young rats, and adult rats were tested on moistened dry-crushed clay with 2000-4000 meter-ohms resistivity; and fruit flies were tested in air. In general, observations were made at the start and at the end of each hour's exposure of the animals to the electric (E-) field to determine whether or not they reacted to the onset and had left the field to enter a region without the field, respectively. A special test was made with fish in which groups of 10 were placed together in a no-field region between equal areas of E-field and no E-field and observed 60 minutes later for location. All tests were performed in the presence of unlocalized magnetic fields of selected magnitudes. Fish visibly reacted in the onset of a 10 and 20 v/m field as did some turtles to a 20 v/m field in tap water. No reaction to E-field onset was observed at 10 or 20 v/m in the other tests. No preferences were observed for or against remaining in the E-fields, except on the part of the turtles which "perceived" the 20 v/m field onset. Ten beagle dogs were tested with ELF current passed through their bodies from leg electrodes to determine their approximate current perception thresholds.

3.0 METHODS

3.1 Introduction

The classical preference method of qualitatively determining the motivational property of an environmental factor

is simple and direct. By offering an organism a free choice between staying in or leaving a spatial region in which a factor is introduced one may, under proper conditions, infer from the results whether or not the factor has aversive or attractive properties. Thus, for example, Overall, et al.(1), have demonstrated that rats spent less time in a chamber exposed to X-irradiation at 1 R/min than in an identical lead-shielded chamber connected to it by an open door. By the use of suitable controls, e.g., a control group without irradiation, as well as randomization of the positions of the irradiated and shielded chambers, it was possible to infer that the rats had a negative preference for X-irradiation. Similar studies have been reported on fish using chemical gradients in water(2). Here a number of fish were tested simultaneously and the number congregating in segments of the gradient were noted. In the present studies, the number of animals located in and out of the E-field at prearranged intervals was used to assess preference for or against the E-field, respectively. In addition, direct gross observation of each test animal was made at the moment an E-field was presented (i.e., when the power switch was turned on) to note any momentary reaction. The occurrence of a startle response or momentary cessation of free movement, under suitable control conditions, was used to infer sensory perception of the E-field. Lack of reaction led to no conclusion.

3.2 Materials

A total of 332 bluegill fingerlings or fry 3-5 cm long, 150 Eastern painted turtles with carapaces 10-16 cm long, 120 three to four week old mallard ducklings, 120 Sprague-Dawley rats 35-40 days old, 120 rats 90-100 days old, and 440 fruit flies (Drosophila melanogaster) three to eight days old were tested. All but the flies were obtained from commercial suppliers. The flies were laboratory bred. Ten beagle dogs later used in a physiology study were tested with leg electrodes.

3.3 Apparatus

Details of the simulation apparatus used to provide the electromagnetic fields and to contain the biological materials are contained in Chapter B of this report. In the present studies the animals were tested in the test stands either on pre-moistened crushed dried clay in two adjacent cells of a 50 x 122 cm water-proof pine box, in water which replaced the clay, or, in the case of the fruit flies, in glass tubes 20 cm

long x 1 cm I.D. with cotton plugs at each end. In each case*, three electrodes were involved in the electric field simulation: the end electrodes were of opposite polarity and the center electrode was given the polarity necessary to produce the electric field in one or the other of the two spaces between. The electrodes were 20 cm apart. When the turtles and ducklings were being tested in water, a brick was set flat on each side of the center electrode with 3.0 cm of water covering. The top of the center electrode was covered by 0.5 cm of water. When the fish were being tested, plastic netting covered the end electrodes to prevent emigration into adjacent cells. The center electrode* was uncovered. Twenty centimeters of water was used in fish tests. In the fruit fly tests, the horizontal glass tube was positioned perpendicular to and through the center electrode which bisected it. The controlled current generators used with the dogs are described in Chapter D.

3.4 Design

3.4.1 - Sets of 15** animals of a given species were randomly divided into five*** groups of three each to be tested in a given replicate of the basic preference test design. The basic design consisted of four EM treatment groups and a control group. Group sizes varied from 15 in one fish group to 30 in the case of turtles tested in water. In general, all groups were tested concurrently.

3.4.2 - Four male and six female beagles were equally divided into two groups. One group was tested with one frequency first, then the other. The other group was tested in reverse sequence.

3.5 Treatments

3.5.1 - Two EM frequencies were tested - 45 Hz and 75 Hz - usually each at two electric field strengths - 10 and 20 volts per meter - and at two respective magnetic field strengths -

---

* A special series of fish tests were made in which four electrodes were used. In these only either the left- or right-hand pair of electrodes were activated with opposite polarities. The fish were placed between the center two electrodes and allowed to migrate at will.

** In the fruit fly study, 11 flies were tested in each tube, one tube per condition per 12 replicates. In the special series of fish tests, 10 fish were placed together in each condition and only eight replicates were tested.

*** Only three groups were tested in the regular fish and the fruit fly studies.

1.0 and 2.0 gauss - concurrently. A control treatment in which only the earth's magnetic field (approximately 0.6 gauss) and the ambient 60 Hz electric field (approximately 0.05 millivolt per meter) were present, was tested. The magnetic fields were not tested in terms of preference; they were ambient for the respective ELF conditions. Fish and fruit flies were tested only at 20 volts per meter except in the special fish tests.

3.5.2 - The dogs were given 2.0-second exposures to 45 Hz or 75 Hz current via leg electrodes in 0.1 mA steps from 0.1 to 1.0 mA.

3.6 Environmental Controls

Each of the three testing rooms was maintained at $75^{\circ} \pm 2$ F during the studies although occasionally the 45 Hz room exceeded this range by 4-6° F. Each room had separate thermostatic control and temperatures were monitored twice daily. Ambient room lighting was equated by photometric measurement. Airflow through the rooms exchanged the air volume 12 times per hour with 100% fresh air. Tap water was used for all aquatic tests except for the fish in the standard two-cell procedure. The demineralized water was used for the latter. Tap water was used for the special fish study (see Section 3.7.3). Water temperature was maintained at 73° F throughout aquatic studies. The observer was situated in a constant position at the start and finish of each test.

3.7 Procedure

3.7.1 - Procedures differed slightly from species to species. However, in general each animal was placed at random in one of the pair of cells and allowed to acclimate for a period of time. Then a series of five tests was given to each as follows:

a. The appropriate electric field was turned on in the cell in which the animal happened to be at the end of its acclimation period.*

b. Sixty minutes later the field was turned off as the position of the animal in respect to the two cells was recorded.

c. If the animal was found out of the field (in the other cell), the position of the field was shifted to the latter cell.*

d. If the animal was found in the original cell (in the field), the position of the field was not shifted.*

---

* In the case of the fruit flies, the E-field was imposed on the half of the glass tube in which the majority of flies were located.

3.7.2 - The fish were acclimated for two hours prior to the initial test. The turtles, ducklings, and rats were acclimated overnight from approximately 4:30 p.m. to 9:30 a.m. The fruit flies were acclimated about one hour. No food was available during acclimation. Water was available in both cells to rats.

3.7.3 - In a special fish study using 10 fish simultaneously in each treatment condition, the randomly selected fish were placed together in the center of three adjacent cells without an E-field being present therein. Either the right- or left-hand cell had an E-field in it. One hour later the distribution of the 10 fish in respect to the three cells was noted. The fish were netted, removed to a temporary holding tank, and the E-field was switched to the opposite end-cell. The fish were replaced in the center cell. One hour later the distribution of the fish was again noted.

3.7.4 - The dogs were placed in a restraining stand (described in Chapter D) which allowed free movement of limbs, head, and trunk with respect to each other. They were in an isolation room and observed from the side through a peep hole. The current increments were separated by 60 seconds and were presented silently. Ten series of ascending presentations were given each dog.

3.8 Observations

3.8.1 - At the beginning of each test in which the E-field was imposed on a cell containing an animal, it was noted whether or not any grossly visible reaction occurred. Preliminary tests with higher strength fields provided instances of the kind of reactions to be expected in each species except fruit flies. Similar observations were made when an E-field was abruptly removed from a cell containing an animal.

3.8.2 - At the end of each 60 minutes the position of the animal(s) with respect to the two (or three) cells was noted. If the animal was on or under a dividing electrode, the fact was noted and that test eliminated from analysis.

3.8.3 - Mortality was noted and, in the case of a dead animal, its previous tests were eliminated from analysis and usually a new animal tested with a control.

3.8.4 - Head, limb, or respiratory movements not normally made during pretest observation were used to infer perception of current if they occurred upon current presentation.

4.0 RESULTS

Table I1 presents the number of times (out of five tests given to each animal or groups of 11 fruit flies) that the animal (or majority of flies) was found to have translocated to the no E-field cell after 60 minutes. Kolmogorov-Smirnov analyses were performed on these data to determine the significance of any differences between (a) any single EM-field group versus its control group, (b) the combined 20-volt per meter groups versus their common control group, or (c) all four EM field groups combined versus their common control group. No differences were obtained which were significant at or less than $p = .05$ using two-tailed tests.

Table I2 presents the data from the special test with bluegill fish in which 10 fish were tested simultaneously in a three-cell arena. Overall, there was no significantly greater tendency for the fish to move into the area without as against with an E-field in it, even though the majority of fish (in the control group, also) moved out of the starting cell. However, when the 20-volt per meter groups were compared with the 10-volt per meter groups, there were more fish found in the E-field than in the bilateral no-field area in the 20-volt per meter groups whereas in the latter there were fewer found in the E-field, ($\chi^2 = 8.06$, df = 1, $p < .01 > .001$). If the fish remaining in the starting cell are included in the analysis as being in the no-field area, the difference is even more significant ($\chi^2 = 11.58$, df = 1, $p < .001$). In no case, however, were the majority of fish found in the E-field.

Table I3 shows the percentage of animals observed to react one or more times to the onset of an E-field. (In the case of the fruit flies, no attempt was made to observe for this since preliminary tests had revealed nothing.) The fish were almost always seen to react to the onset of 20-volt per meter fields whenever they were more than 45° inclined from the plane of the electrodes. A few turtles tested in water were observed to react to the onset of the 20-volt per meter fields only. No reactions were observed in the other animals which could be related to E-field onset. The fish used in the special study were grossly observed at its conclusion by switching the E-fields onto those found in the no-field cells. One or more fish always reacted to both 10- and 20-volt per meter fields. No further attempt was made to quantify this observation.

An analysis was made of the translocation frequencies of the turtles which were observed to react one or more times to the 20-volt per meter field onsets versus those which were not seen to react. Dichotomizing these data into those translocating fewer than twice versus those translocating 2-5 times in five tests, yielded $\chi^2 = 4.59$ which with one degree of freedom had a probability of less than 5% ($p < .05$) of occurring by chance.

Since nearly all the fish were observed to react to the onset of the 20-volt per meter field, the above analysis could not be made. On those tests in which a reaction was observed, only 41.9% resulted in a translocation.

Three dogs clearly reacted to one or both frequencies at current values of from 0.7 to 1.0 mA. After one or two reactions, all ceased to react to up to 1.0 mA (upper limit of equipment).

5.0 DISCUSSION

On the basis of the results obtained, it would appear that a 10-volt per meter electric field in water or in conductive soil did not create an environment which these small animals found noxious enough to leave, though 3-5 cm bluegill fry could perceive it. A 20-volt per meter E-field could be perceived by some turtles (and probably could be by other aquatic animals of comparable size) and those that did perceive it tended to leave it. That fish which perceived a 20-volt per meter field did not tend to leave it more often than chance (41.9% versus 50%), probably can be understood as due to the fish being able to reduce the head-to-tail potential difference by turning 90° away from the field lines, i.e., by turning parallel to the plane of the electrodes(3). Since the fish is long and narrow, this maneuver is effective; it is less effective for the turtle.

The presence of the nonlocalized, uniform magnetic fields during the preference tests might have had effects on the activity levels of the animals and thus affected the validity of the tests. However, while the ducklings exposed to magnetic fields made somewhat fewer translocations than the control ducklings, the difference did not approach significance at the 5% level. The same applied to the fruit flies. In the other animal tests, no consistent pattern emerged. Thus, the magnetic fields did not appear to affect activity level.

In the regular fish study, it was observed that many of the individually tested fish located themselves under and collinear with the center electrode rods. Since it could not be ascertained whether they had translocated, no datum could be recorded for the test during which they so positioned themselves. Similarly, it was not possible to impose the E-field on the fish in this position, nor, for that matter, on fish so positioned under end electrode rods. Thus, in this study, a good number of tests had to be discarded as invalid. Replacement fish were tested (with concurrent controls) to obtain a minimum of 15 per group.

The special fish study was undertaken both to test some fish at 10 volts per meter and to test congregated fish, whether schooling or not. It was hoped that the positioning tendency described in the previous paragraph would be lessened by congregational tendencies. Such, indeed was the case. Another reason for this study was to determine whether these fish would tend to stay out of a region containing a 10-or 20-volt per meter field as opposed to leaving an E-field imposed on them. The results did not indicate any tendency to stay out of the E-fields. However, it is questionable whether a strong conclusion should be drawn from these results. A fish could enter the region containing the E-field at an angle less than 45° from the electrode plane and, thus, not have perceived the field until it turned back beyond the 45° oblique. In this case, a fish, if it perceived the field, would be likely to behave as did the fish in the regular preference study, i.e., it might as often remain in the field as leave it.

The dog current threshold study was insufficient to establish a threshold. It was clear, however, that below 0.7 mA, ELF current originating at leg electrodes was generally insufficient to elicit overt responses in the beagle. It was also evident that below 1.0 mA most reactive beagles tested adapted quickly and either suppressed overt reactions or did not perceive the current.

The general conclusion drawn from the preference studies was that an electrical field of 10 volts per meter in either a conductive or nonconductive medium was insufficiently noxious to promote a translocational movement in any of the animals tested. Larger fish(3) and other larger aquatic animals may be expected to be more sensitive to E-fields than the small animals tested in these studies. Therefore, a 10-volt per meter gradient in water may be close to the upper limit for a noneffective gradient.

6.0 APPENDICES

6.1 Tables of Data

Table I1 - Frequencies and mean number of translocations observed out of five tests per animal exposed to EM fields under conditions permitting escape from the E-field by translocation

CONDITION	TRANS-LOCATIONS	SUBJECTS: DUCKLINGS N=120	FISH* N=119	FLIES* N=60**	RATS (YOUNG) N=120	RATS (ADULT) N=120	TURTLES (H_2O) N=150	TURTLES (DIRT) N=135
Control	0	13	4	3	8	14	12	17
	1	4	13	0	10	7	2	4
	2	3 M=0.96	13 M=1.93	3 M=2.25	4 M=1.00	2 M=0.58	12 M=1.33	5 M=0.67
	3	3	7	4	2	1	2	0
	4	1	4	1	0	0	2	1
	5	0	1	1	0	0	0	0
45 Hz 1 gauss 10 v/m	0	16	1	2	17	19	14	10
	1	5	5	4	1	5	9	9
	2	1 M=0.58	8 M=1.83	2 M=1.75	4 M=0.67	0 M=0.21	6 M=0.80	5 M=1.09
	3	1	4	3	1	0	1	2
	4	1	0	1	1	0	0	1
	5	1	0	0	0	0	0	0
45 Hz 2 gauss 20 v/m	0	15	3	2	11	20	8	11
	1	4	7	6	8	4	7	9
	2	5 M=0.58	7 M=2.15	1 M=1.50	1 M=0.92	0 M=0.16	4 M=1.83	6 M=0.89
	3	0	4	2	4	0	5	1
	4	0	2	1	0	0	5	0
	5	0	3	0	0	0	1	0
75 Hz 1 gauss 10 v/m	0	13	1	3	19	19	13	16
	1	4	5	2	3	4	6	6
	2	4 M=0.88	10 M=1.83	2 M=1.75	1 M=0.33	1 M=0.25	8 M=1.17	3 M=0.67
	3	3	1	5	1	0	2	2
	4	0	0	0	0	0	1	0
	5	0	1	0	0	0	0	0
75 Hz 2 gauss 20 v/m	0	[illegible]	1	3	12	11	13	16
	1	7	4	2	2	7	8	7
	2	5 M=0.83	7 M=1.80	4 M=1.58	6 M=1.17	5 M=0.84	4 M=1.10	4 M=0.5[illegible]
	3	1	3	3	2	1	1	0
	4	0	0	0	2	0	1	0
	5	0	0	0	0	0	2	0

* [illegible]ested at 20 v/m instead of 10 v/m

** [illegible]ixty squads of 11 flies each

Table I2 - Mean number of 10 fish found in respective cells at the end of 60-minute tests using squads of 10 fish per treatment simultaneously

POSITION	CONTROL N=80	45 HZ 10 V/M, 1.0 GAUSS N=80	45 HZ 20 V/M, 2.0 GAUSS N=80	75 HZ 10 V/M, 1.0 GAUSS N=80	75 HZ 20 V/M, 2.0 GAUSS N=80
In field cell	3.00*	1.88	2.75	1.75	4.38
In start cell	4.25	5.25	4.50	5.38	4.00
In no-field cell	2.75**	2.87	2.75	2.87	1.62

* Left-hand cell
** Right-hand cell

Table I3 - Percentage of animals visibly reacting to the onset of E-fields in standard tests

SUBJECT	45 HZ 10 V/M	45 HZ 20 V/M	75 HZ 10 V/M	75 HZ 20 V/M
Ducklings	0	0	0	0
Fish	-	88.7	-	93.9
Rats, young	0	0	0	0
Rats, adult	0	0	0	0
Turtles/dirt	0	0	0	0
Turtles/water	0	13.3	0	16.7

6.2 References

1. Overall, J. E., Logie, L. C., and Brown, W. L., Radiation Research, 11, 589-599, 1959.

2. Shelford, V. E., Laboratory and Field Ecology, Baltimore: Williams & Wilkins, 1929.

3. Fishing with Electricity, R. Vibert, Ed., Fishing News (Books) Ltd., by arrangement with The Food and Agriculture Organization of the United Nations, 1967.

CHAPTER J
RAT AVOIDANCE LEARNING STUDY

William B. Coate

TABLE OF CONTENTS

1.0 INTRODUCTION

Chapter J contains the methodology and results of a rat avoidance learning study performed in fulfilling the requirements of the Naval Electronic Systems Command, Contract No. N000-39-69-C-1572. The objective of this contract was to assess preliminarily the possibilities of biological/ecological impact from the continuous presence in a given locale of electric fields, magnetic fields, and earth return currents of specified magnitudes. The present study was performed as a pilot study to determine whether gross effects on learning ability could be expected from prenatal and preweaning exposure to these levels of electromagnetic (EM) fields and currents at extremely low frequencies (ELF).

2.0 SUMMARY

Young albino rats, offspring of male and female parents exposed to EM fields or control conditions for at least 80 days prior to conception and during gestation and raised in the EM fields to weaning, were tested at 30-32 days postnatally for speed of acquisition of an active shock-avoidance response. The number of trials required to attain a criterion of five successive avoidance responses was the main dependent variable. No significant effect was obtained either on this variable or on trials to an escape criterion.

3.0 METHODS

3.1 Introduction

A number of environmental agents applied prenatally, neonatally, or both have been shown to affect the ability of young rats to learn in a variety of situations. X-irradiation at moderate and low levels in single doses retarded learning of a simple maze whether given to the test animals _in utero_(1, 2) or within six days postnatally(3). This effect has been regarded as a manifestation of a direct or indirect radiation effect on the ontogenetic development of the nervous system. On the other hand, data have been reported(4) which show that in an avoidance learning task, prenatally X-irradiated rats learned faster

than controls. This effect has been attributed to reduction in emotionality (fear) by prior X-irradiation(5). Regardless of the explanations of the obtained effects, if effects on behavioral adaptiveness can be demonstrated as due to earlier exposure to an ELF electromagnetic environment, a potential ecologically important phenomenon will have been revealed. The ecological implications would depend on the nature of the effect obtained.

3.2 Materials

Eighty-seven male and 101 female offspring of 43 female Sprague-Dawley rats were used in this study at 30-32 days of age. All were apparently healthy and active pups. The pups were derived from parents exposed to ELF and control conditions for 80 days prior to mating. The pregnant females and the resulting litters were similarly exposed until the pups were tested.

3.3 Apparatus

Details of the simulation apparatus used to provide the electromagnetic environments and to contain the animals during exposure are contained in Chapter B of this report. The avoidance testing apparatus used in the present study consisted of five identical automated one-way shock-avoidance boxes operated by an electromechanical programmer which timed all essential intervals (Figure J1). Each box contained a grid floor which was connected to a 60 Hz 190-volt shock source through a 950,000 ohm current-limiting resistor in series with the animal. A 10 rpm synchronous motor drove a small "safety platform" through a 90° arc to dump the rat onto the grid at scheduled intervals. The platform was returned to horizontal, immediately after attaining the vertical, by tension-spring followed by gravitional action.

3.4 Design

Five treatment groups were randomly distributed among the five shock-avoidance boxes and, as nearly as possible, were equally distributed throughout a three-week testing period. Whenever possible, i.e., when enough 30-32 day old pups were ready for testing on a given day, all five avoidance boxes were employed concurrently and all five treatments were concurrently represented. Pups were selected for testing at random as they reached the required age.

3.5 Treatments

Two EM frequencies were imposed - 45 Hz and 75 Hz - each at two magnetic field strengths - 1.0 gauss and 2.0 gauss - and at 20 volts per meter concurrently. The voltage gradient appeared in a moistened dry-crushed clay substrate on which the rats lived throughout treatment and thus the animals were subjected to unmeasured body currents as a function of their size and position on the substrate as well as of their total body resistance. The resistivity of the substrate was maintained between 2000 and 5000 meter-ohms. A control treatment, in which only the earth's magnetic field (approximately 0.6 gauss) and the ambient 60 Hz electric field (approximately 0.05 millivolt per meter) were present, was tested. The 45 Hz treatments were applied in one room, the 75 Hz treatments in another, and the control in a third. The treatments were continuous, 23 hours a day, throughout the gestation period and through weaning, except that the 75 Hz treatments were interrupted for one seven-day period and for two one-day periods during the 42-day total gestation span. The seven-day shutdown came at the beginning of parturition and overlapped the first trimester (the period of organogenesis) in 46 of the pups tested.

3.6 Environmental Controls

Each of the three treatment rooms was maintained at 76 ± 2° F throughout the breeding phase. Each room had separate thermostatic control and temperatures were monitored twice daily. Ambient room lighting was equated by photometric measurement. An 9 hour on-15 hour off light cycle was maintained. Airflow through the rooms exchanged the volume 12 times per hour with 100% fresh air. Testing was conducted in a separate laboratory room.

3.7 Procedure

3.7.1 - Male and female rats approximately 130 days old were mated in the respective treatment conditions by placing one male with two females for a maximum period of 21 days. Each female was abdominally palpated at seven and 14 days to ascertain whether or not she was pregnant. Pregnant females were "isolated" from other rats until parturition occurred. "Isolation" consisted of being mechanically and visually separated but not completely out of physical contact with the occupant of the adjacent cage(s). "Isolated" dams reared their unculled litters

alone. Food and water were available ad libitum. No nesting material was supplied other than the crushed clay substrate. Pups were weighed 24 hours, four days, and 21 days after birth; otherwise, they were not handled until the day of testing.

3.7.2 - Each animal tested was first placed on the "safety platform" in an avoidance box for 60 seconds. At the end of this period, the platform was activated to dump the rat onto the grid floor and to start the first trial. Three seconds later 0.2 mA shock was delivered through the grid for a maximum of five seconds. If the rat attained the safety platform within the three seconds prior to shock onset, an avoidance response was scored. If the animal was in contact with the grid when the voltage was turned on but reached the platform before it was terminated, an escape response was scored. The presence of voltage on the grid was signalled to the observer by a red pilot light above the boxes. The five boxes were operated one at a time in the same sequence until each rat received a maximum of 50 trials. If all five rats attained the criterion of five successive avoidance responses prior to 50 trials, the session was terminated. The intertrial interval for each box was 60 seconds. All rats were tested between 9:00 and 11:45 a.m.

3.8 Observations

On each trial, each rat was scored as either making an avoidance response, making an escape response, or missing an escape response. If the rat was in the midst of climbing onto the safety platform when the shock was presented, the observer noted whether or not a shock-induced reaction was elicited. If not, an avoidance response was scored. Two rats managed to maintain a posture on the grid floor which allowed them to avoid shock altogether without ever reaching the safety platform. These animals' data were eliminated.

4.0 RESULTS

4.1 Avoidance Learning

Table J1 presents the distribution and median number of trials required by each treatment group to reach the criterion of five successive avoidance responses. A median test for five

independent samples yielded $\chi^2 = 1.62$, df = 4, p >.80. A similar two-group test in which all the EM field exposed rats were compared to the control rats yielded $\chi^2 = .40$, df = 1, p >.50. A final statistical test was made in which the 45 Hz groups combined were compared to the control group ($\chi^2 = .44$, df = 21, p = >.50). This test was made to exclude the questionable 75 Hz animals whose exposures were incomplete. None of these differences were statistically significant.

4.2 Escape Learning

The median number of trials required to achieve five successive escape* responses was 5.0 for all groups combined. Table J2 presents the distribution and median number of trials to this criterion for each treatment group. Inspection of the data indicated that no significant differences among the groups would be found, with or without inclusion of the questionable 75 Hz data.

5.0 DISCUSSION

There was in the results of this study no suggestion of any effect on avoidance learning ability from EM exposures at ELF. The five groups tested all yielded quite similar percentage frequency distributions for trials to avoidance and escape learning criteria.

The shutdown of the 75 Hz EM fields during the critical developmental phase of organogenesis of so many (46) of the pups tested probably invalidated that portion of the study. However, it should be noted that the median trials to the avoidance learning criterion for those rats whose EM-field exposure included the period of organogenesis was exactly the same (11.0) as was this median for those lacking this portion of the exposure. Similarly, Table 1 shows the similarity of the total 75 Hz group to the total 45 Hz (and control) group. Thus, there appears to be no reason to believe that the interruption of the 75 Hz fields effectively altered the functional environment as far as brain or other organic development goes.

---

* Avoidance responses were counted as escape responses in this tabulation on the assumption that the rat would have escaped had he not avoided the shock.

6.0 APPENDICES

6.1 Tables and Figure

Table J1 - Number/percentage of rats in each group requiring the indicated number of trials to reach five successive avoidance responses

		TRIALS TO CRITERION (AVOIDANCE)									
GROUP	N	5-10	11-16	17-22	23-28	29-34	35-40	41-46	47-50	50+*	MDN
Control	47	25	14	4	0	1	1	0	0	2	10.14
		53%	30%	9%	0%	2%	2%	0%	0%	4%	
45 Hz, 1 gauss 20 v/m	32	14	13	1	0	1	0	0	0	3	11.43
		44%	41%	3%	0%	3%	0%	0%	0%	10%	
45 Hz, 2 gauss 20 v/m	29	15	10	3	0	0	0	0	0	1	10.32
		52%	35%	10%	0%	0%	0%	0%	0%	4%	
75 Hz, 1 gauss 20 v/m	41	18	14	4	3	0	0	0	0	2	11.57
		44%	34%	10%	7%	0%	0%	0%	0%	5%	
75 Hz, 2 gauss 20 v/m	37	20	10	3	0	1	0	0	0	3	10.05
		54%	27%	8%	0	3%	0%	0%	0%	8%	

* Animals which failed to attain the criterion

Table J. - Number (percentage) of rats in each group requiring the indicated number of trials to reach five successive escape or avoidance responses

GROUP	N	TRIALS TO CRITERION (ESCAPE) 3-7	8-12	13-17	18-22	23-27	28-32	33-37	38-42	50+*	MDN
Control	47	38	11	0	1	0	1	1	0	2	6.51
		81%	22%	0%	2%	0%	2%	2%		4%	
45 Hz, 1 gauss 20 v/m	32	21	10	1	0	0	1	0	0	1	6.79
		66%	31%	3%	0%	0%	3%	0%	0%	3%	
45 Hz, 2 gauss 20 v/m	29	21	6	1	0	0	0	0	0	1	6.38
		73%	21%	4%	0%	0%	0%	0%	0%	4%	
75 Hz, 1 gauss 20 v/m	41	30	5	3	1	0	0	0	0	2	6.29
		73%	12%	7%	2%	0%	0%	0%	0%	5%	
75 Hz, 2 gauss 20 v/m	37	29	3	2	0	0	1	0	0	2	6.08
		78%	8%	5%	0%	0%	3%	0%	0%	5%	

* Animals which failed to attain the criterion

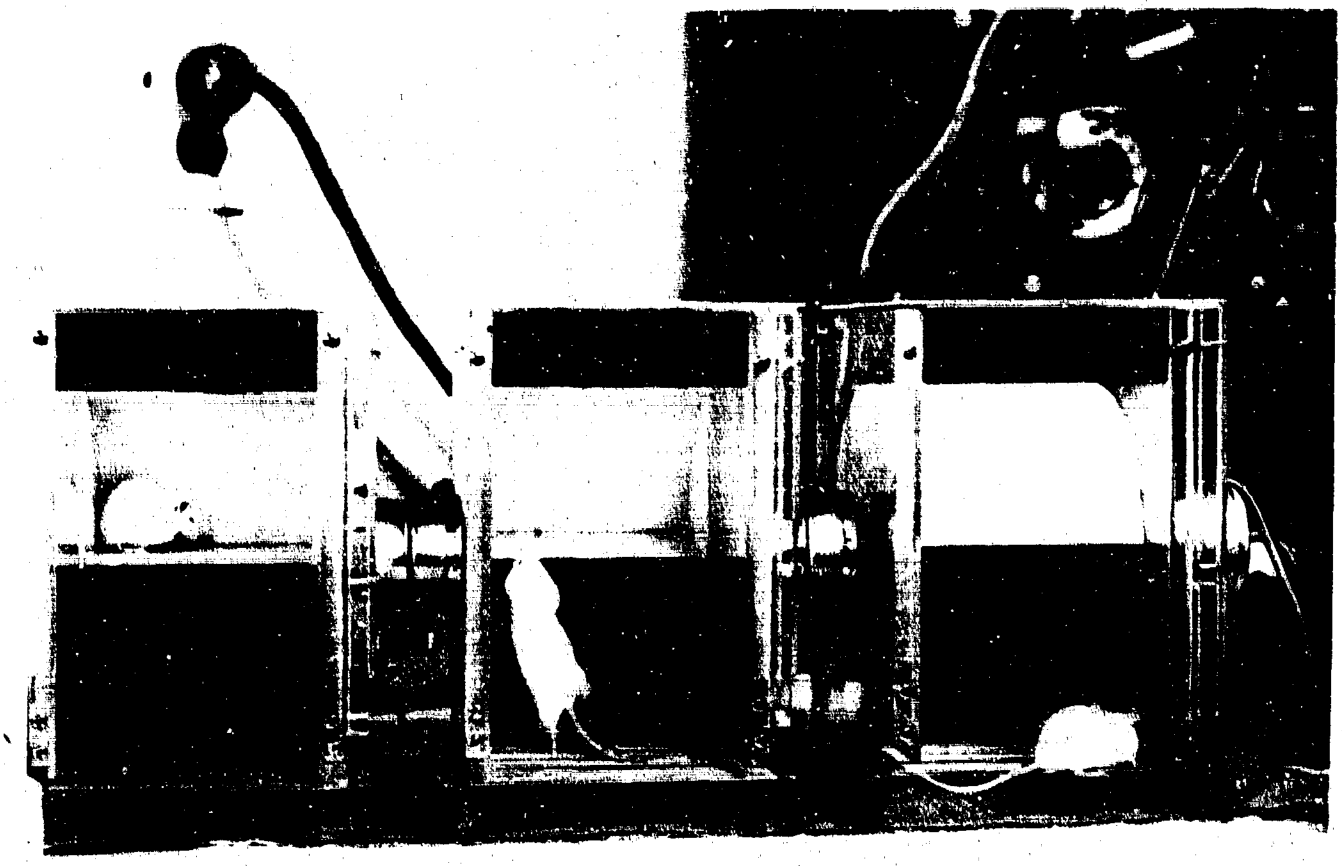


Figure J1 - Three rat avoidance boxes shown in three phases of operation from left to right: (1) between trials, (2) during avoidance or escape periods, and (3) just before the start of avoidance period (safety platform not yet returned to horizontal) shown with mice

6.2 References

1. Furchtgott, E., Magill, E., and Openshaw, J. W., J. comp. physiol. Psychol., 51, 178-180, 1958.

2. Kaplan, S. J., in Response to the Nervous System to Ionizing Radiation, Haley, T. J., and Snider, R. S., Eds., New York: Academic Press, 1962.

3. Levinson, B., and Zeigler, H. P., J. comp. physiol. Psychol., 52, 53-55, 1959.

4. Furchtgott, E., and Wechkin, S., J. comp. physiol. Psychol., 55, 69-72, 1962.

5. Joffe, J. M., Prenatal Determinants of Behavior, Braunschweig: Pergamon Press, 1969, p. 52.

CHAPTER K
SITE BRAVO SOIL ORGANISM STUDY

Jack M. Heinemann
William B. Coate

TABLE OF CONTENTS

1.0 INTRODUCTION

Chapter K presents the procedures used and the results obtained in an on-site study of soil organisms at Bravo Test Facility-I (BTF-I) near Clam Lake, Wisconsin (Figure K1), in fulfilling the requirements of the Naval Electronic Systems Command, Contract No. N000-39-69-C-1572. The objective of this contract was to assess preliminarily the possibilities of biological/ecological impact from the continuous presence in a given area of electric fields, magnetic fields, and earth return currents of specific magnitudes. This study was designed to see if the soil arthropod and worm population adjacent to a ground terminal of the antenna array would be affected by the operation of a SANGUINE test system. Photographs of surrounding vegetation were also taken as a preliminary means of assessing gross changes in plant growth in the area.

2.0 SUMMARY

Soil arthropods and worms were extracted from soil samples in a plot near a ground terminal and from a control plot prior to initial operation of BTF-I and one year following during which it was in operation. Animals were identified and counted to establish community structures. Major taxonomic groups from each year were compared to see if any significant changes had occurred in number of animals in these groups in the one-year period during which BTF-I was in operation. Panoramic photographs were made at the two plots in order to assess grossly any changes in vegetation. Significant reductions occurred in the four major taxons in the test plot and in three of these in the control plot. No detrimental changes in vegetation were noted.

3.0 METHODS

3.1 Introduction

Soil arthropods number 10^4 -10^5 per square meter in forest soils and provide a convenient animal population for studying changes in community structure such as ecological succession.

3.2 Apparatus

The Tullgren funnel technique was used to extract soil arthropods and several other taxa from soil samples. A diagram of the portable apparatus is shown in Figure K2.

This is a dynamic, dry-sample extraction procedure which uses a 25-watt light bulb to produce heat, light, and dessication that drive organisms down through a core sample of soil until they drop from the sample into a vial of preservative. Coring of the soil and leaf litter was done with a tin cylinder having a cross-sectional area of 38 square centimeters forced into the soil to a depth of approximately six centimeters. Samples were wrapped in cheesecloth and placed in closed plastic containers for transport to the BTF-I laboratory where six Tullgren apparatuses were located for extraction.

3.3 Design

Two sampling sites were selected - a test plot adjacent to the south ground terminal (40°30' N; 90°37' W) and a control plot located approximately eight miles from any of the ground terminals (41°08' N; 91°08' W). Due to right-of-way clearance in the forest, it was necessary to work 10 feet away from the location of the ground terminal in order to obtain undisturbed forest soil for the test plot. The control plot was selected on the basis of a forest stand similar to the test plot, adequate distance from ground terminals, and safety from lumbering during the subsequent year. A false ground terminal trench was prepared at the control site to simulate the disturbance caused by placing the ground terminal system that runs next to the test plot. Each plot was 10 feet square and transected by four equally spaced sampling lines parallel to the ground terminal trench.

Twenty samples were obtained from each plot each year, five samples being selected at random along each transect. First sampling was on 2 July 1969; the subsequent samples were obtained on 1 July 1970. Both sampling areas were roped off and marked in order to prevent accidental movement of equipment across them.

3.4 Treatments

The antenna was not supplied with current until after the sampling was completed in 1969. During the following year electronic operation and testing of the Sanguine system was carried out; however, information regarding the field strengths and frequencies used for this testing schedule have not been made available to Hazleton Laboratories. Therefore, the treatment being studied is the cumulative effect of all testing during a one-year period.

3.5 Environmental Conditions

No effort was made to control any environmental parameter at either site, except an attempt to prevent accidental intrusion by men and equipment. There was no evidence of entry to either site. The ropes and stakes were intact on 1 July 1970. Climatic conditions did vary over the sampling period (1969-1970). The weather prior to sampling in 1969 was characterized by heavy snowfall and cold, damp conditions; temperatures in June-early July 1969 ranged from lows of 45° F to highs of 75° F. A milder winter preceded sampling in 1970 and climatic conditions in late June-early July 1970 were warm and dry with temperatures ranging from lows of 60° F to highs of 95° F. Samples were extracted under nearly identical conditions each year since they were performed at the laboratory at BTF-I.

3.6 Procedure

Twenty samples were removed from each site without disturbing soil texture and animal burrows in the samples. Each sample was wrapped in a double layer of cheesecloth and inverted in a plastic container which was sealed. Samples were taken to the mitigation laboratory within two hours and placed in an inverted position on a screen within the extracting apparatus. Extraction began simultaneously on all samples and continued for a fixed period of 48 hours. Animals were collected in small vials of 80% ethanol which were then capped. Coded samples were shipped to Dr. D. A. Crossley, consultant at the Institute of Ecology, University of Georgia, Athens, Georgia, who performed the identification and counts of the soil animals extracted each year without knowing the source of any sample.

3.7 Observations

On-site observations consisted on 360° panoramic photographs of each sampling area to document the nature and density of plant growth and BTF-I construction for use in subsequent evaluations. Segments of these photographs which show only the sampling plots are found in Figures K3-K6. Tullgren apparatuses were checked frequently during the 48-hour extraction period to insure that animals were being collected properly and that the equipment was operating properly.

4.0 RESULTS

Table K1 presents the quantitative distribution of soil arthropods and annelids collected during two yearly samplings at BTF-I. Data are expressed in terms of numbers per square meter (M^2). Percentage changes in numbers of the four major taxa, which comprised 93-97% of the total animals extracted each year, are shown in Table K2. The total number of animals obtained decreased in the test plot by 65.3% from 1969 to 1970 as compared to a decrease of 31.5% for the control plot during the same period. Percentage decreases were greater in the test plot for the four major taxa over this period as compared to percentage decreases for these same groups in the control plot.

The reductions in numbers of individuals in each classification were analyzed by the Mann-Whitney U-test(1) for independent samples, using a two-tailed test. The criterion of significance chosen was the .05 level of probability. The reductions in all three Acarina families were highly significant ($p < .01$) for the test plot. Only the Prostigmata and Mesostigmata were significantly reduced in the control plot ($p < .02 > .01$ and $p < .05 > .02$, respectively). The Collembola were reduced in both plots significantly ($p < .01$ and $p < .02 > .01$ for test and control plots, respectively).

Table K3 shows the percentage change in numbers of each of the four major taxa which were obtained from each of the four lines parallel to the ground line in the test plot each year. The lines were approximately 10, 13.3, 16.6, and 20 feet from the ground line.

Figures K3 and K4 show the control site in July 1969 and July 1970, respectively. The cover in this area consists of (1) good stocking of northern hardwood of pole and saw timber stand size and (2) poor stocking of aspen-paper birch of pole timber stand size. Figure K5 and K6 show the test plot in July 1969 and July 1970, respectively. The cover here consists of (1) good stocking of aspen-paper birch of pole and saw timber stand size and (2) poor stocking of northern hardwoods of pole timber stand size. From a subjective viewpoint, normal growth was found in vegetation of the forest floor, understory, and canopy in both the test and control areas in July 1970. Grasses and fern were beginning to grow along and on the backfilled antenna trenches in both areas. Forest floor vegetation seemed more luxuriant in both areas in 1970 compared to 1969. In Figures K5 and K6 growth of sprouts over a one-year period can be seen on the sunlit tree at the right side of the picture. Early discernible growth is also observed on the two conifers immediately to the left of that tree.

5.0 DISCUSSION

The primary objective of this study was to determine the relative abundance of soil arthropods in a test plot adjacent to a ground terminal of the antenna array and in a control plot and compare these community structures with samples collected the following year. The initial sampling in both areas showed a normal tendency of arthropod communities to contain many orders of low abundance and a few orders of high abundance. The subsequent sampling in 1970 presented a very similar community structure, except for a statistically significant reduction in the numbers of animals at the test plot in all four taxa which made up 97.6% of the population in July 1969 and 95.9% of the population in July 1970. In the control plot for the same two periods, these four taxa comprised 92.2% of the population (1969) and 95.4% (1970). It is evident from data shown in Table K2 that some factor or factors exerted an influence which produced a general decrease in the sampled population abundance in both test and control plots. Climatic conditions prior to sampling in July 1970 were much hotter and dryer than before the initial sampling in July 1969. This would tend to decrease populations in the upper portions of leaf and soil litter. While it was hoped that each sampling plot would be acted upon by identical extrinsic and intrinsic forces, except for the electrical fields and earth return currents in the vicinity of the test plot from the BTF-I ground terminal, the sites were not under continuous surveillance between samplings. The possibilities of some uncontrolled factor(s) having caused the greater decrease in population in the test plot makes it difficult to conclude that operation of BTF-I was responsible for the observed effect. For example, due to the cleared right-of-way adjacent to the test plot, the amount of direct sunlight was greater on this plot than on the control plot during the period after July 1969. Furthermore, it is quite possible that the greater decrease in Acarina in the test plot was simply a consequence of the fact that it had a much higher population at the outset and that a generally adverse climate may have a greater impact on a densely populated area if competition for food and optimal living space is greater than in a similar low density area. In order to rule out these kinds of artifacts, it is necessary either (a) to find more similar plots to sample which show comparable initial distributions of populations or (b) to have historical evidence of stability despite changes in climate, etc. Neither course was open for this study due to time, budget, and/or geographic limitations. Finally, the fact that there was no gradient of population reductions correlated (inversely) with distance from the ground terminal lends no support to a conclusion that operation of BTF-I impacted the test plot.

6.0 APPENDICES

6.1 Tables and Figures

Table K1 - Quantitative distribution of soil invertebrates

TAXONS EXTRACTED	NUMBERS PER SQUARE METER: TEST PLOT 1969	TEST PLOT 1970	CONTROL PLOT 1969	CONTROL PLOT 1970
Acarina - prostigmata (mite)	13,237	4,618	5,028	3,263
Acarina - mesostigmata (mite)	6,526	974	3,158	1,618
Acarina - oribatei (mite)	46,039	15,974	18,907	16,947
Collembola (springtails)	26,408	9,908	11,496	5,539
Coleoptera (beetles, weevils)	290	265	346	356
Hymenoptera (ants, wasps, bees)	238	41	41	26
Protura	146	199	96	119
Diptera (true flies)	146	94	138	93
Hemiptera (true bugs)	66	81	13	27
Thysanoptera (thrips)	26	213	138	250
Corrodentia (book lice)	13	41	-	67
Homoptera (aphids)	13	107	-	106
Pseudoscorpiones (false scorpions)	53	15	96	53
Araneoe (spiders)	146	80	180	158
Chilopoda (centipeds)	26	26	28	-
Diplopoda (millipeds)	79	-	-	-
Pauropoda	79	-	69	39
Annelida	461	92	1,069	13
TOTAL	94,454	32,820	41,878	28,687

Table K2 - Quantitative distribution of four main taxons of extracted soil arthropods

TAXON		MEAN (M) NUMBER & STANDARD DEVIATION (S.D.) OF ARTHROPODS PER BORING — TEST PLOT 1969	TEST PLOT 1970	TEST PLOT % CHANGE		CONTROL PLOT 1969	CONTROL PLOT 1970	CONTROL PLOT % CHANGE
Acari -								
Prostigmata	M =	50.3	17.6	-65.1	M =	19.1	12.4	-35.1
	S.D. =	37.4	11.1		S.D. =	10.95	8.3	
Mesostigmata	M =	24.8	3.7	-85.1	M =	12.0	6.2	-48.8
	S.D. =	19.1	3.9		S.D. =	11.8	8.09	
Oribatei	M =	175.0	60.7	-65.3	M =	71.8	64.4	-10.4
	S.D. =	92.9	44.1		S.D. =	71.6	36.8	
Collembola	M =	100.4	37.7	-62.5	M =	43.7	21.1	-51.8
	S.D. =	64.2	26.2		S.D. =	26.2	11.1	

Table K3 - Percentage change in numbers of individuals between July 1969 and July 1970 in relationship to distance from a ground terminal

TAXA EXTRACTED / TRANSECT AND DISTANCE FROM GROUND TERMINAL	PERCENT CHANGE IN NUMBERS A 10'	B 13.3'	C 16.6'	D 20'
Mites				
Prostigmata	-62.9	-76.9	-41.2	-67.2
Mesostigmata	-82.2	-93.0	-53.8	-87.1
Oribatei	-60.1	-76.8	-47.0	-69.5
Springtails				
Collembola	-71.5	-78.7	+ 2.2	-68.7

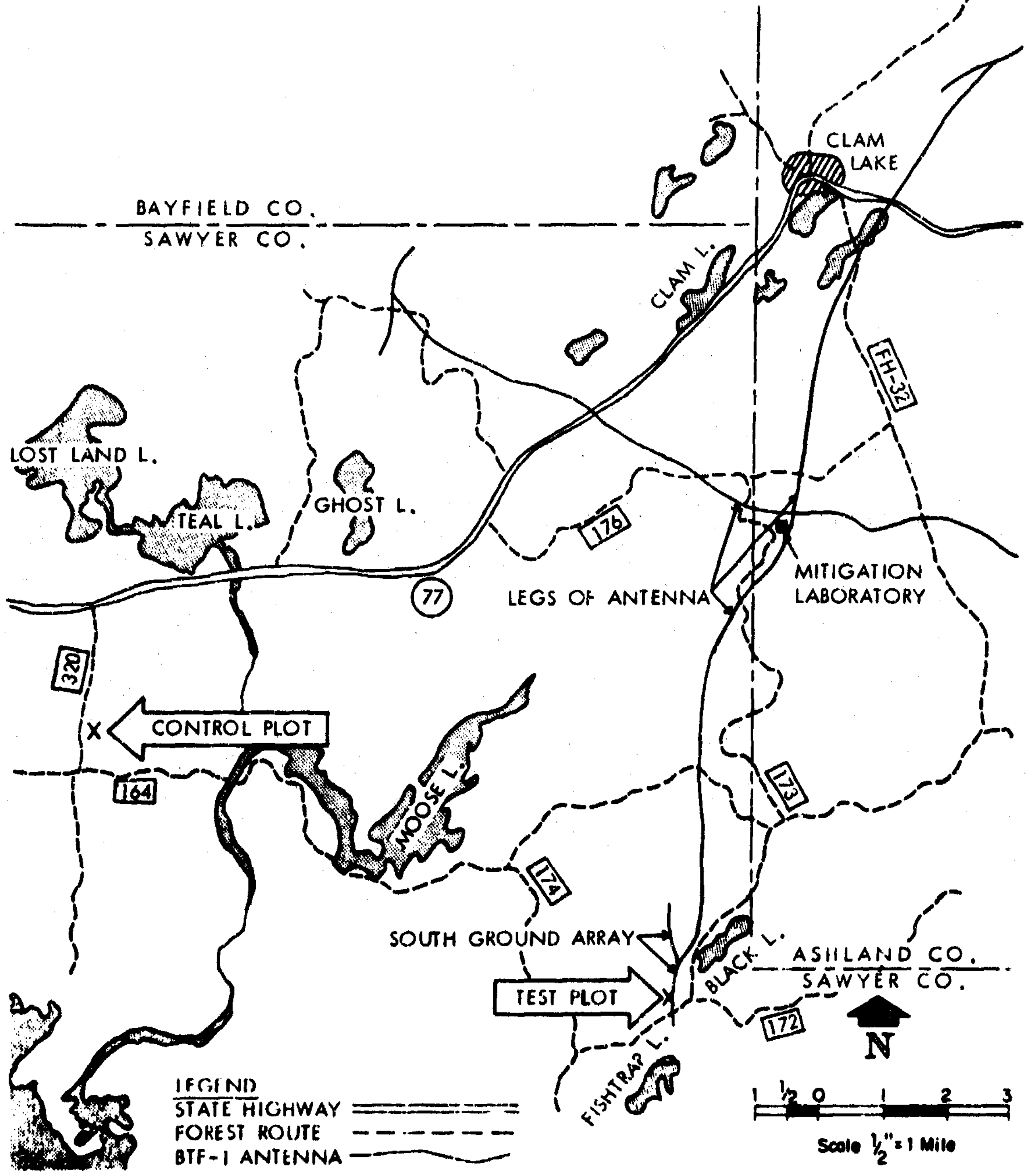


Figure K1 - Map of Bravo Test Facility-I Clam Lake, Wisconsin, showing location of soil invertebrates sampling plots

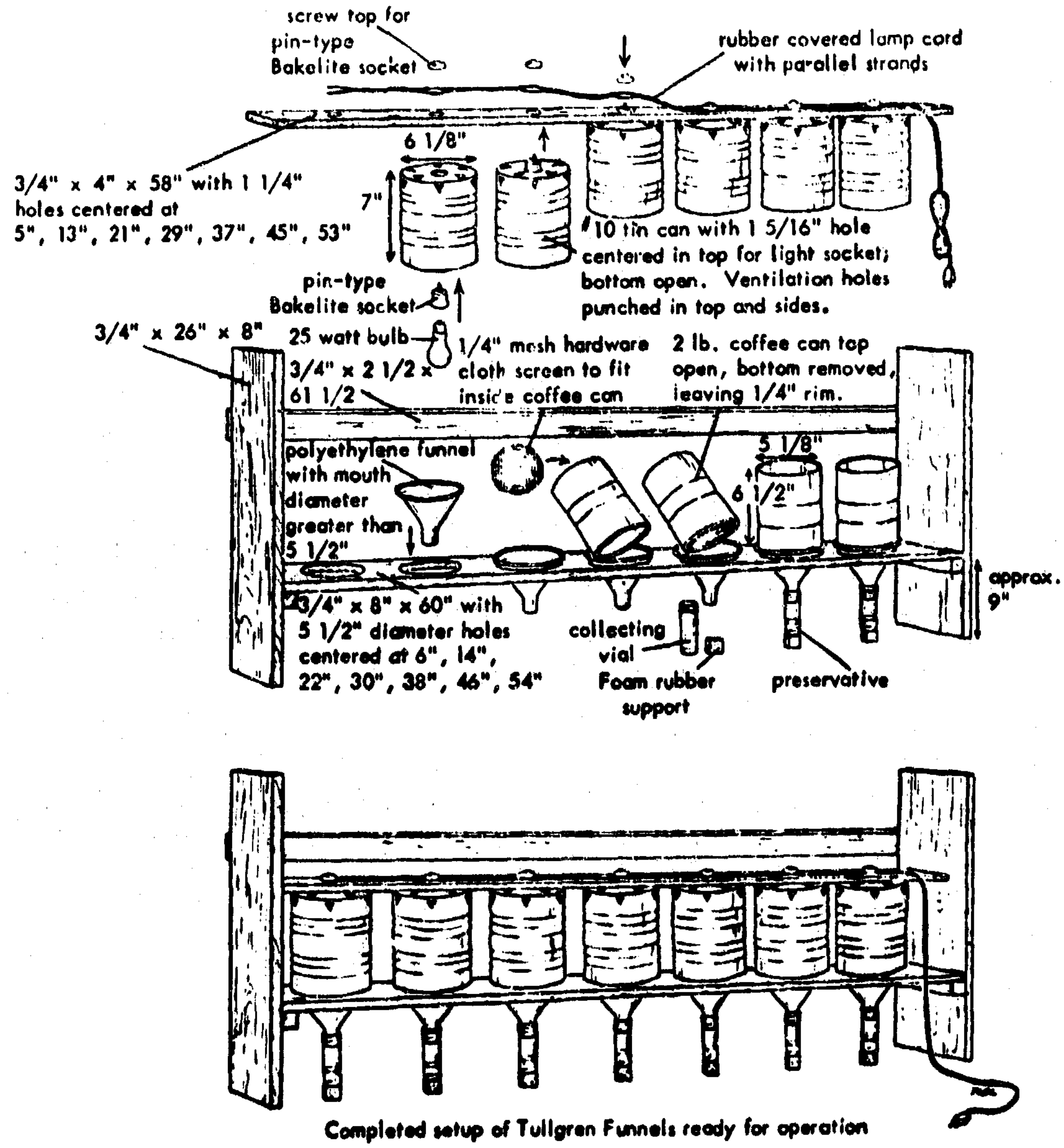


Figure K? - Construction of portable Tullgren funnel extraction apparatus (design of Dr. K. K. Bohnsack, San Diego State College)

Figure K3 - View of control plot, July 2, 1969 (41°8' N; 91°8' W)

Figure K4 - View of control plot, July 1, 1970 (41°8' N; 91°8' W)

Figure K5 - View of test plot, July 2, 1969 (40°30' N; 90°37' W)

Figure K6 - View of test plot, July 1, 1970 (40°30' N; 90°37' W)

6.2 Reference

1. Mann. H. B., and Whitney, D. R., Ann. math. Statist., 18, 50-60, 1947.

CHAPTER L

GENERAL CONCLUSIONS

1.0 INTRODUCTION

This section presents conclusions based on the results of the studies reported in previous chapters and draws certain implications from them regarding SANGUINE system design criteria. Both conclusions and implications are those solely of the contractor.

2.0 CONCLUSIONS

In general, it may be concluded that little or no evidence was obtained to indicate that a SANGUINE system would harm biota throughout its environment. Since the results of the majority of the studies were negative at EM field levels well above those likely to be associated with a SANGUINE antenna, only the positive findings will be further discussed.

No firm conclusion should be drawn from the on-site study of soil organisms. The initial differences between the control and test plot were too great to permit a valid interpretation of difference in population decline between the two areas.

A 10-volt per meter ELF electrical field gradient in natural fresh water is likely to present a hazard to fishes as a direct function of their length. A 20-volt per meter gradient was found to be aversive to turtles as small as 10-16 cm in length. Fish as small as 3-5 cm in length reacted to the onset of both gradients but did not appear to find them aversive enough either to stay out of or leave them. It would, therefore, seem reasonable to conclude that, where possible, the gradient in water should be held to a value below 10 volts per meter in order to avoid ecological impact. No effects on behavior were seen from a gradient as high as 20 volts per meter in conductive soil or in air. Therefore, it may be concluded that a 10-volt per meter gradient at the system grounds would not drive small terrestrial animals away.

The question then follows as to whether small animals in the region of a 10-volt per meter gradient in the earth would be adversely affected by prolonged exposure. No effects were observed in mammals up to the size of adult rats. Dogs, on the other hand, were found to have slightly elevated blood pressure after as little as one week of exposure to this field in combination with a 2.0 gauss magnetic field and 0.5 mA body current for 16 hours a day, five days

per week. This finding would suggest that 10 volts per meter could be too high an earth conducted gradient for animals as large as a beagle dog with total resistance in the region of 2000 ohms. These dogs did not visibly react to the onset of 0.5 mA applied to the lower legs.

While not a conclusive finding, the growth retardation observed in sunflower seedlings also suggests that a 10-volt per meter soil-borne gradient and/or a 1.0 gauss magnetic field may be somewhat above the safe limit for plant life.

The results of the three mutagenic studies are difficult to interpret. It was expected that the bacteria and onion root-tips would have been more sensitive to mutagenesis than the fruit flies, inasmuch as the former were exposed to electric fields in a conductive medium and the latter in air. However, the former were unaffected, while a highly significant increase in the frequency of a mutagenic indicator was found in the fruit fly experiment. At the present time it is deemed prudent to defer drawing a firm conclusion from these results until sufficient confirmatory experiments have been made to assess possible artifactual explanations of the present results. The likelihood of 20-volt per meter, 2.0 gauss EM fields in the ELF range having a strong mutagenic effect is remote. No biophysical basis for such a possibility is presently known. Furthermore, if ELF fields of this low magnitude could have such an effect, the existence of even higher fields associated with power frequency generators and transmission systems should have produced evidence of mutagenesis. No such evidence has ever been reported.

CHAPTER M

RECOMMENDATIONS FOR FUTURE RESEARCH

1.0 INTRODUCTION

The recommendations herein are those based on the results of the studies conducted on this contract only. There are other areas of biological/ecological research which perhaps should be explored, but which are not directly related to the present work.

2.0 RECOMMENDATIONS

The most obvious needs are to conduct parametric studies designed to determine, separately, safety limits for the ELF electrical and magnetic fields. For instance, other seeds should be germinated and grown in fields of both higher and lower magnitude than in the present studies. Similarly, perception and preference thresholds should be established for fish, turtles, and perhaps other aquatic animals such as otter. These studies are needed to set electrical field design criteria for system grounds. Since 10 volts per meter now looks to be on the high side, studies should be conducted at one, five, and 10 volts per meter with sufficient sample sizes to provide a good empirical basis for setting the criterion.

Secondly, an expanded fruit fly mutagenic study should immediately be conducted to confirm or disconfirm the results of the present study. The implications of these results are too important to be neglected. Again, should the results be confirmed it would be necessary to separate magnetic and electric fields to pinpoint the source of the effect. Dose-response studies should then also be conducted to determine the form of the dose-response relationship.

Thirdly, it is recommended that a careful study be made of blood pressure, the electrocardiogram, and body temperature in dogs exposed only to ELF body current up to 0.5 mA or to a 1.0 or 2.0 gauss magnetic field. Remote recordings should be made while the dogs are exposed, with before and after exposure tests, in order to ascertain the degree of transitoriness of any changes observed. Recordings could be made using either telemetry or hard-wire technique. A minimum six-week exposure period is recommended.

Finally, because aquatic organisms are so intimately in contact with a voltage gradient appearing in water, it would be important to investigate possible reproductive effects in an aquatic animal.

A suggested model is the frog which may be studied from the fertilized egg through metamorphosis to adult frog development. The threshold for any effect obtained at 10 volts per meter should be determined.

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)

HAZLETON LABORATORIES, INCORPORATED
A Subsidiary of TRW, Inc.
P. O. Box 30
Falls Church, Virginia 22046

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

N/A

3. REPORT TITLE

PROJECT SANGUINE BIOLOGICAL EFFECTS TEST PROGRAM - PILOT STUDIES

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final Report, May 1969 - November 1970

5. AUTHOR(S) (First name, middle initial, last name)

William B. Coate
Robert E. Kimball
William O. Negherbon
Smith Sae Hoo
Richard A. Pledger
Frederick E. Reno
Winnie R. Teeters

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
November 30, 1970	147	16

8a. CONTRACT OR GRANT NO.

N000-39-69-C-1572

b. PROJECT NO.

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)

NAVELEX FR70

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT

Distribution of this document is unlimited

11. SUPPLEMENTARY NOTES

Qualified requesters may obtain this report from DDC

12. SPONSORING MILITARY ACTIVITY

Naval Electronic Systems Command
Headquarters
Washington, D. C. 20360

13. ABSTRACT

Pilot laboratory experiments were conducted to assess preliminarily the possibility of effects on biota from exposures of various durations to electric fields of 10 and 20 volts per meter and magnetic fields of 1.0 and 2.0 gauss at two extremely low frequencies, 45 and 75 Hz. In some experiments the electric field was set up in a conductive medium (moist soil, water, or nutritive medium) and in others in the air In one study, 0.5 mA current at these frequencies was passed through the bodies of dogs concurrent with the application of the fields. The laboratory studies consisted of the following with possible electromagnetic effects indicated, if any:

- Rat Fertility Studies - no effects
- Rat Avoidance Learning Study - no effects
- Canine Physiology Study - possible elevated blood pressure and rectal temperature
- Insect Mutagenic Study - possible dominant lethal mutation rate increase
- Bacteria Mutagenesis Study - no effects
- Plant Cytogenetic Study - no effects
- Seed Germination and Early Growth Study - possible growth inhibition in one of three species
- Multiple-Species Electric Field Perception and Preference Study - no preference effects except in a few turtles which reacted to the onset of 20 v/m fields. Fish "perceived" the onset of 10 v/m fields.

An on-site census of soil arthropods near a ground terminal of the Wisconsin Test Facility before and after power-on operation was inconclusive.

DD FORM 1473 1 NOV 65 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

UNCLASSIFIED
Security Classification

KEY WORDS	LINK A ROLE	LINK A WT	LINK B ROLE	LINK B WT	LINK C ROLE	LINK C WT
Biological Effects						
Electric fields						
Magnetic fields						
Extremely Low Frequency (ELF)						
Body current						
Step potential						
Laboratory studies						
On-site field study						